ACID AND ALKALINE PHOSPHOMONOESTERASES IN NORMAL AND TUMOUR-BEARING CHICKENS, AND IN CERTAIN SPONTANEOUS, VIRUS ASSOCIATED AND CHEMICALLY INDUCED CHICKEN TUMOURS. A HISTOCHEMICAL AND BIOCHEMICAL STUDY.

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C O N T E N T S.

I. Introduction.

Phosphatases are a group of enzymes capable of hydrolyzing esters of phosphoric acid with the production of inorganic phosphate.

A phosphatase was first detected in certain cereals by the Japanese workers Suzuki, Yoshimura and Takaishi (1907). Since then, many different varieties of phosphatases have been found to occur in practically all living cells, both in plants and in animals.

The whole subject of phosphatases was comprehensively reviewed from both the specifically enzymatic and the general biological viewpoints by Folley and Kay (1936). A few years later, Albers (1940) reviewed the subject again, covering the enzymological aspects with special thoroughness. Moog (1945) later published a review dealing with the physiological significance of this group of enzymes.

Folley and Kay (1936) classified phosphatases into several main groups according to — (a) substrates attacked, and (b) pH activity curves. The ordinary alkaline and acid phosphomonoesterases belong to the class AI (pH optimum about 9) and AII (pH 5). This classification appears to be fundamentally sound, although Cloetens (1939), on the basis of work with partly purified preparations, has divided the first group into two types with slightly different properties, and Gomori (1943b) has verified Folley and Kay's prediction that there was a separate hexose-diphosphatase.

Recently/

Recently, Abolins and Abolins (1949) suggested that there are at least two different acid phosphatases (one opt. pH 6.6 and another pH 5.5 - 5.0) in the anterior pituitary of the guinea-pig.

Many workers have attempted to differentiate types of alkaline phosphatases but the results are contradictory. Bodansky (1937) reported that various bile salts may decrease the activity of bone and kidney phosphatases but not of intestinal phosphatase. Cloetens (1939), on the other hand grouped bone and intestine together in contrast to kidney on the basis of relative content of alkaline phosphatase 'I' and 'II'. Schmidt and Thannhauser (1943) found that the highly purified intestinal phosphatases are completely insensitive to bile salts. Bowers, Outhouse and Forbes (1940) reported that when amino-ethyl phosphate was used as substrate, purified faecal phosphatase differs from kidney phosphatase in its response to Mg^{++}, and this difference was not present when B-glycerophosphate was used; so the authors suggested that new substrates might be of value in typing phosphatases.

One of the most interesting researches in phosphatases is that of Albers, Beyer, Bohnemkamp and Muller (1938), who found that the phosphomonoesterases consist of a 'co-phosphatase' (co-enzyme) and an 'apo-phosphatase' (carrier). They separated by dialysis the co-enzyme and carrier of acid top yeast phosphatase and of kidney phosphatase. On recombining the co-enzyme of one with the other, they produced two new enzymes

which/

which showed the pH activity of their presumably protein components. Substrate preference, since it was the same for both original enzymes, thus appeared to be a function of the co-enzyme.

Much work has been done on the subject of activators and inhibitors which influence the function of phosphatases. Bamann and Heumüller (1940) have investigated the relation of many divalent cations to the activity of liver alkaline phosphatase, and have found that Mn^{++} have a greater accelerating effect than Mg^{++}, which was classically supposed to be the specific activator. Cloetens (1941) reported that the kidney alkaline phosphatase complex involves two metal ions, one, easily dialysable, the other, which is probably zinc under normal conditions, is removed only with difficulty. Hove, Elvehjem and Hart (1940) found that although zinc often acts as a depressant, it increases the activity of crude intestinal phosphatases, yet decreases it after dialysis, and autolysis of mucosal tissue produces a zinc co-activator, whose function can be imitated by any pure α-amino acid. Massart and Dufait (1942) found that alkaline phosphatase was a metal protein inhibited by cyanide but not by aldehyde or ketone reagents. Albers (1940) reported the retarding action of various amino acids on alkaline phosphatase from kidney. Williams and Watson (1940) found glycine had a similar action on bone phosphatase. Moog (1944) observed a marked increase in the histochemical reaction for acid phosphatase when 0.01 M. ascorbic acid was added to the medium.

As mentioned earlier the physiological functions of phosphatases have been thoroughly reviewed by Moog in 1945. These may be summarized as follows:-

(a) Calcification. Gomori (1943a), as a result of his histo-chemical study of the developing bones of chick and mammal embryos, concluded that all calcifying cartilage contained alkaline phosphatase, and the deposit of calcium salts only occurred in the active region; phosphatase negative regions did not calcify. Moog (1944) in a day to day study of chick embryo development showed that the moderate phosphatase activity of the mesenchymal rudiment of the long bones disappeared as cartilage differentiation proceeded, but reappeared with augmented activity in the perichondrial zone as the cartilage cells hypertrophied. Engel and Furuta (1942) and Gomori (1943a) demonstrated a correlation between alkaline phosphatase and calcification in developing teeth. Woodard and Kenney (1942), through the deposition of radio-active phosphorus, found that the fixation rate of P^{32} was in positive correlation with the phosphatase content in several types of tumours. Blum (1944) reported the acceleration of calcification in experimental fractures in rabbits following the introduction of alkaline phosphatase into the damaged region. Bourne (1943) obtained similar results by intramuscular injection. Gutowska et al (1943) reported a low content of phosphatase in the shell gland, oviduct and ova of the laying hen by biochemical assay. However, Bourne (1943), by histochemical study, showed the location of phosphatase to

be/

be on the cuticular border of the oviducal epithelium. The highest concentration was found in the shell gland.

Gomori (1943a), using histochemical methods, showed that concentric rings of calcification succeeded similar zones of alkaline phosphatase activity in tubercular lesions in the lung of the rabbit. Breedis, Florey and Furth (1943) and Wachstein (1944) observed calcification of phosphatase containing necrotic tissue and cellular debris in experimentally damaged kidneys of rats and rabbits.

Huggins (1931) reported the ossification of canine bladder epithelium in the rectus sheath. Gomori (1943a) by histochemical methods extended this observation using other tissues and transplantation sites. He found that only when bladder epithelium was transplanted to rectus sheath did ossification result. He suggested that it involved the specific induction of a complete calcifying system specific to the rectus sheath.

Gomori (1943a) reported the absence of phosphatase in calcifying hyaline connective tissue, as for example in arteriosclerosis, pyelonephritis, and calcifying goitre. Woodard and Kenney (1942) found that in skeletal tumours, ossification did not occur even with liberal supplies of phosphatase. In rickets (Freeman and McLean, 1941; Gomori, 1943a), it was found that though sufficient phosphatase was present both in the serum and in osteoid tissue normal calcification was blocked. Thus it seems that the presence of phosphatase does not necessarily bring about calcification. Conversely, pathological calcification may occur without phosphatase.

(b)/

(b) Transportation. Lundsgaard (1933) first put forward the hypothesis that phosphatase plays a role in the reabsorption of glucose in the kidney tubules. Though the original method was later criticized by certain workers (Lundsgaard, 1935; Lambrechts, 1937; Kritzler and Gutman, 1941), the hypothesis is still regarded as correct. Moreover, subsequent histochemical work by Gomori (1941a) and Bourne (1943) in a variety of mammals, by Kabat and Furth (1941) in man and mouse embryo, and by Moog (1944) in chick embryos, showed that alkaline phosphatase activity is highest in the brush borders of the proximal convoluted tubules. On the basis of this work, Wilmer (1944) offered the tentative explanation that glucose molecules are captured from the glomerular filtrate through phosphorylation, whilst adenylpyrophosphate supplies the energy for the formation of the ester linkage; at a later stage the hexose monophosphate is dephosphorylated by phosphatase and sent back to the blood.

Although acid phosphatase is also fairly rich in the renal tubules, its function is still not clear. Wachstein (1944) reported that in cortical destruction caused by choline deficiency, both acid and alkaline phosphatases survived in reduced amount. According to Beck (1942), kidney acid phosphatase is much more sensitive to phlorizin than is alkaline phosphatase.

Gomori/

Gomori (1941a), Kabat and Furth (1941) both described the localization of alkaline phosphatase in the intestinal epithelium. Cera and Bellini (1940) found that the addition of glycerophosphate to olive oil may increase the rate of absorption of the oil in rats. Bourne and Mackinnon (1944) reported that the phosphatase is localized in a zone between the nucleus and the lumenal border of the intestinal epithelium, and Emmel (1945) has produced evidence that the enzyme is localized in the Golgi zone. All these facts suggest that phosphatase may play a role in the transport of glucose and fatty acid molecules through the intestinal mucosa.

(c) Growth and Differentiation. Lipman (1936) reported the presence of alkaline phosphatase in chick embryo extracts. The activity of the enzyme reaches a peak at six days and another at fifteen days. Kroon et al (1940) reported a correlation between phosphatase and phosphorus content in the skeleton during development and linked the enzyme with phosphate fixation. Moog (1943, 1944) demonstrated histochemically both acid and alkaline phosphatases in the cytoplasm and nuclei of chick embryos as early as the primitive streak stage. Both enzymes exist in all undifferentiated embryonal tissues until differentiation commences. As soon as the primitive tissues differentiate to their definitive form and functional condition, the enzymes either disappear or accumulate at specific locations. As a result of this Moog suggested that the occurrence of phosphatases in the embryo may be diphasic, those of the primitive stage being perhaps active agents in the/

the chemistry of differentiation, and those of the functional stage may be themselves consequent on differentiation. Lindeman (1949) studied the alkaline and acid enzyme activity of embryonic chick retina and concluded that the alkaline enzyme activity increases slowly up to the 16th day and then more rapidly to the 19th day when it reaches its maximum: after the 20th day it begins to decrease until three days subsequent to hatching, at which time it establishes a level of activity which is apparently constant throughout the bird's life; the acid enzyme activity is considerably higher than the alkaline at the 12th day, but shows only a slight increase up to 19th day, after which it also falls to a new level. Since the rise in activity of the two enzymes corresponds to the period of the cellular maturation, it seems to lend support to the concept that they may be associated with the histochemistry of differentiation. Yao (1949) reported that during Drosophila embryogenesis, acid phosphatase showed no change of activity, whereas alkaline phosphatase activity rises only after the contraction of the germ band, increases in activity and then decreases again.

Fell and Danielli (1943) found that in the healing of cuts and burns in rat skin, a marked increase of alkaline phosphatase activity occurred in the regenerating connective tissue after 5 or 6 days. As healing progressed the enzyme content decreased. Bourne (1943) and Bourne and Mackenzie Mackinnon (1944) reported a similar phenomenon in regenerating bone.

Bodian/

Bodian and Mellors (1944) found a relation between acid phosphatase and chromatolysis in nerve cells of rhesus monkeys. They showed that shortly after cutting the axons the acid enzyme activity increased markedly in the cytoplasm where chromatolysis was occurring, and it remained high until the regeneration of Nissl's granules.

Edlbacher and Kutscher (1931) reported that in various kinds of malignant tumours of chicken, rat and man, there is an enzyme capable of dephosphorylating both plant and animal nucleic acids. The enzyme could also be detected in benign tumours but to a lesser degree. Wienbeck (1933) and Macfayden (1934) corroborated this finding and demonstrated two enzymes, one a nucleotidase and the other a phosphatase, in extracts of chicken tumours. Morii and Takabatake (1932) reported that alkaline phosphatase is present in human uterine carcinoma metastatic in lymphnodes, but not in leiomyomata. Horii (1933) reported a diminution of alkaline phosphatase in carcinomata after irradiation. According to Waldschmidt-Leitz and Köhler (1933), Uramoto (1932), and Köhler (1934), the decreasing amount of parenchymatous tissue and the parallel increase of necrotic tissue in the progressive ageing of tumours, is accompanied by a gradual declination of all phosphatase activity. Köhler (1934) found that with maturation the alkaline phosphatase activity of tumour tissue, muscle and kidney decreased, and the kidneys of tumourous rats and cancer-immune Wistar rats contained more active enzyme than kidneys from normal rats. He also showed that muscle and kidney

?decline

phosphatase/

phosphatase of healthy rats was fully activated with $MgCl_2$ whilst that of cancerous rats was only about 30% activated. The blood enzyme of his cancerous rats was not only higher than normal but its autoactivation was nearly doubled. Schoonover and Ely (1935) found that the red blood cells of cancer patients showed a higher phosphatase activity at pH 5.0 to 6.0 than those from normal people of the same sex, but after adding Mg^{++} both showed the same level again. They suggested that the additional phosphatase activity of cancerous blood may be due to the presence of an 'acid' phosphatase which is not activated by Mg, and which is either absent in normal red blood cells, or present only in small quantities.

Kabat and Furth (1941) studied the histochemical distribution of alkaline phosphatase in several types of chicken, mouse, rat and human tumours. Landow, Kabat and Newman (1942) Wolf, Kabat and Newman (1943) studied both alkaline and acid phosphatases in tumours of the nervous system. Greenstein (1942) determined biochemically the content of the two enzymes in a long list of normal and neoplastic tissues, which he summarized at a later date (1943a, b). He concluded that generally speaking, it is very difficult to determine a simple correlation between neoplasia and the activity of the phosphatases. It seems that the activity of both acid and alkaline enzymes is unaffected or somewhat decreased with the onset of neoplasia. Sure, et al (1935) reported that with the large numbers of rats bearing Walker carcinosarcoma No. 256 the blood serum phosphatase is slightly lower than

normal/

normal controls on the same diet. Greenstein (1947) suggested that the range of activity of phosphatase in tumourous tissues is narrower than in normal.

In hepatoma however, the acid phosphatase activity, high in normal liver, is found to be almost doubled; the alkaline phosphatase activity is ordinarily little changed except in butter-yellow-induced hepatoma of the rat, in which a markedly high content is associated with bone formation, (White and Edwards, 1942). Kabat and Furth (1941) reported that there was no evident change in alkaline enzyme content in the above mentioned type of tumour, and no calcification was ever detected even in necrotic tissue. Gomori (1941b) reported a high acid phosphatase activity in numerous human gastric carcinomata. Greenstein (1942) reported that the normally high content of alkaline phosphatase in the intestinal mucosa of rats and mice had practically disappeared in adenocarcinoma of this viscus.

Kabat and Furth (1941) showed that in osteogenic sarcoma of the chicken, the osteoblasts usually contained an increased amount of alkaline phosphatase when cartilage or bone formation was taking place. Woodard (1942) indicated that in metastatic osteogenic tumours secondary to carcinoma of the prostate the alkaline phosphatase activity is high and the acid phosphatase activity is even higher than in the primary tumour.

The object of the present work is to investigate the difference in distribution between acid and alkaline phosphatase in/

in the tissues of healthy chickens, to ascertain whether there is any significant variation in distribution or concentration of the phosphatases between healthy chickens and those bearing tumours, and to compare them with the above mentioned findings.

II. Materials.

All the normal adult fowls and the six-week-old chicks used for tumour induction or transplantation were obtained from the special tumour susceptible strain of Brown Leghorns at the Poultry Research Centre, Edinburgh.

Material for histochemical study was obtained from two healthy six-week-old chicks (one male and one female) one healthy laying hen, ten chickens bearing Rous sarcoma No. 1, two chickens bearing the transmissible Campbell fibrosarcoma, (virus associated), five chickens bearing dibenzanthracene induced fibrosarcoma (GRCH/15), and other fowls affected with spontaneous neoplasmata were obtained from various sources.

The female six-week-old healthy chick was injected intravenously with lithium carmine (5 ml daily for 5 days) in order to differentiate the macrophages and the reticulo-endothelial system.

The healthy laying hen was used for particular study of the reproductive organs. Pieces of tissue were taken from the ovary and from different parts of the oviduct and fixed for further use.

Rous/

Rous sarcoma No. 1 tumours were induced by injecting a saline suspension of freeze-dried material intramuscularly into six-week-old Brown Leghorn chickens. After 2 or 3 weeks when the tumours were well established at the sites of injection (the routine sites were the right leg and the left breast of the bird), they were killed by dislocation of the neck.

Rous tumours were also induced in five birds by intravenous injection of a suspension of fresh tumour cells in saline. All of them developed numerous tumours in the lung, whilst some showed wide-spread tumours in the voluntary muscles, myocardium, meninges, thymus, pancreas, proventriculus, gizzard, duodenum and ilium, and one formed an ulcerated tumour in the neck. From these birds, therefore, many organs containing tumours were obtained for study.

The transmissible Campbell's fibrosarcoma was induced in the same way as the Rous I sarcoma, with tumour dessicates. The percentage of successful takes was much less than with the Rous and no metastatic tumour was ever found.

The dibenzanthracene induced fibrosarcoma (GRCH/15) was propagated by intramuscular transplantation from bird to bird. Small pieces of tumour tissue were obtained by biopsy and then implanted into the breast muscle of six-week-old chicks with a sterilized syringe and a wide bore hypodermic needle. The tumour usually grew to a considerable size within two to three weeks and exhausted the hosts. It was frequently accompanied with liver cirrhosis, which phenomenon will be commented upon later. Metastasis often occurred in the lungs, liver, pancreas

and/

and proventriculus. The metastatic tumours were taken together with the surrounding tissues for study.

A black Minorca hen bearing a spherical benign fibrous tumour measuring 3 cms. in diameter under the right wing was obtained from a local farm. The bird was killed and material taken for transplantation, but with negative results. The original tumour was, however, used for histochemical examination.

A histiocytic sarcoma was kindly sent to the laboratory from the Poultry Laboratory of the Ministry of Agriculture and Fisheries, Eskgrove, Lasswade, Midlothian. Transplantation was attempted but again with negative results, possibly because the tumour, which occurred on the wing of a young chick was stored in the refrigerator overnight, before implanting.

A spontaneous ovarian adenocarcinoma was obtained from a Brown Leghorn hen at the Poultry Research Centre. A hard intra-abdominal mass could be detected by palpation. The bird was killed by dislocation of the neck. A portion of the ilium was extensively thickened through tumourous infiltration in the wall. Several small, hard nodules resulting from transcoelomic implantation were found in the mesentery and pancreas. One from each site was taken for study.

A spontaneous lymphoid tumour arising from the Bursa of Fabricius of a cross-bred hen was kindly supplied by the Poultry Department of the Royal (Dick) Veterinary College, Edinburgh. The bird was killed by dislocation of the neck and fresh tumour tissues were immediately collected and

fixed/

fixed in chilled acetone. Tumours were also found on the mesentery, ilium and proventriculus wall.

A spontaneous fibro-leiomyoma was occasionally found in the ventral ligament of the oviduct of a Brown Leghorn laying hen killed for other experiment by dislocation of the neck. Fresh tumour tissue was immediately collected and fixed for histochemical study.

III. Methods and Results.

A. Histochemical Study.

The original method for the histochemical demonstration of alkaline phosphatase in tissue sections was published independently by Gomori (1939) in America and by Takamatsu (1939) in Japan. It depends upon the hydrolysis of the substrate (sodium β-glycerophosphate) through the action of the enzyme in the presence of calcium chloride with the formation and precipitation of calcium phosphate at the site of enzyme activity. This substance may then be converted to black cobalt sulphide. The slide is finally counter-stained as desired, and the result is a clear picture of the actual localization of the enzyme. This method has been critically studied by Danielli (1946) and proved to be quite specific and suitable for cytological study.

For acid phosphatase, Gomori (1941b) produced a somewhat similar technique but differing from that for alkaline phosphatase in the substitution of lead nitrate for calcium chloride/

chloride, since calcium phosphate is soluble at acid pH. The lead phosphate precipitated is visualized through conversion to lead sulphide. Wolf, Kabat and Newman (1943) have modified this method by using β-glycerophosphate instead of the α-salt, and by lengthening the maximum time of incubation from 15 to 48 hours.

Recently, Gomori (1949) published another report emphasizing the specificity of the above two methods. He used 19 different substrates for comparison and found that glycerophosphate was the best for both acid and alkaline phosphatases, and chilled acetone was the best fixative for acid phosphatase and was also suitable for alkaline phosphatase.

Manheimer and Seligman (1948) and Seligman and Manheimer (1949) have published two new methods for alkaline and acid phosphatases which utilize organic phosphoric esters to mark the site of enzyme activity. Although these methods have the advantages of the avoidance of heavy metals, in the marked insolubility of the diazonium dyes produced, and even in the possibility of demonstrating both acid and alkaline phosphatases in the same tissue section, they have not so far given results comparable to the simpler Gomori technique.

Wang and Grossman (1949) reported better results obtained by using a freezing-drying method for the histochemical study of alkaline phosphatase instead of the ordinary paraffin embedding method.

Ruyter and Neumann (1949) stated that incubation of

sections from which the paraffin wax had not been removed gave better results for the histochemical demonstration of alkaline phosphatase. This method was tried but it was found that sections were easily detached from the slide and the result was not dependable because of non-specific heavy tissue absorption of cobalt sulphide. The methods for the histochemical demonstration of alkaline and acid phosphatase finally adopted and used throughout the present investigation were as follows:-

Pieces of tissue no more than 3 mm thick were fixed in chilled acetone and kept in a refrigerator at $0^{o}C.$ for 12-24 hours. They were dehydrated in two changes of acetone at room temperature for 12-24 hours. Clearing was carried out in two changes of benzene each for $\frac{1}{2}$ hour. The tissues were then embedded in paraffin wax (m.p. $56^{o}C.$) in a vacuum paraffin embedding oven for 2 hours. Sections were cut at 5μ immediately after the blocks were made. From every tissue block used in this work, five serial sections were taken: one for ordinary haematoxylin-eosin staining, one for acid phosphatase, one for acid phosphatase control, one for alkaline phosphatase, and one for alkaline phosphatase control. They were flattened by floating on warm distilled water, stuck on to clean cover glasses with a thin layer of egg albumen, dried at room temperature for 1-3 hours, and then passed through two changes of xylol, followed by acetone and distilled water. Then they were put into a Petri dish with tissue sections upward and submerged in the substrate, which was made up as follows/

follows:-

(1) For acid phosphatase:

0.1 N acetate buffer solution pH 5.0	12.5 ml.
5% lead nitrate solution	5.0 ml.
Distilled water	75.0 ml.
2% sodium β-glycerophosphate (B.D.H.)	15.0 ml.

The solution was made up in the above order, kept in the refrigerator for 12-24 hours, and filtered before use. The sections were incubated for 18-20 hours at 37°C. Control sections were incubated in a similar solution in which the sodium β-glycerophosphate had been omitted, being replaced by an equal volume of distilled water.

After incubation they were then washed in 1% acetic acid for a ½ minute and dipped quickly into two changes of distilled water. Ammonium sulphide (6 drops in 100 ml distilled water) was then used to convert the precipitated lead phosphate to black lead sulphide. After washing twice with tap water the sections were counter-stained with 1% eosin, and were then taken up through the alcohols to xylol, and mounted in Canada balsam as quickly as was consistent with efficient dehydration and clearing.

(2) For alkaline phosphatase:

2% sodium barbitone solution	20.0 ml.
2% calcium chloride solution (crystals)	10.0 ml.
2% magnesium sulphate solution	10.0 ml.
2% sodium β-glycerophosphate (B.D.H.)	20.0 ml.
Distilled water	40.0 ml.

(The final pH of the above mixture is approximately 9.4 the sodium barbitone serving as a buffer)

Control/

Control sections were incubated in a similar solution in which the glycerophosphate had been substituted by an equal volume of distilled water. The incubation time was 12-16 hours at 37°C.

After incubation, the sections were washed and placed in 1% calcium chloride solution for 10 minutes, transferred to 2% cobalt nitrate for 10 minutes, washed quickly in 5 changes of distilled water for one minute, treated with ammonium sulphide (6 drops in 100 ml of distilled water) for 5 minutes, washed again in tap water, counter-stained with eosin, passed through alcohols to xylol as rapidly as possible, and finally mounted in Canada balsam.

The histochemical results for both normal and tumourous tissue are given in some detail. A certain amount of repetition being inevitable the results are also summarised in tabular form for convenience of reference, at the end of this section.

HISTOCHEMICAL RESULTS.

(1) The distribution of acid phosphatase.

(a) In the tissues of normal chickens.

"Stained", in the following descriptions means the formation of lead sulphide at the site of enzyme activity.

Central Nervous System. Generally speaking, all the nerve cells and fibres were rather deeply stained throughout the brain and spinal cord. The oligodendroglia showed the most/

most intense reaction followed by the microglia, the neurons, and the astrocytes in that order. The Purkinje cells however, exhibited only slight nuclear staining. The dendrites and axons of the very deeply impregnated neural elements could at times be followed for some distance. Within the larger nerve cells, particularly in the motor neurons, the neuro-fibrils stood out sharply, and could be traced for some distance into the cell processes. (Fig. 1.). The nuclei of ependymal cells were deeply stained, the cytoplasm only slightly. The leptomeninges and chorioidal cells showed moderate staining. The nuclei, but not the cytoplasm, of the endothelial cells forming the blood capillaries were lightly stained.

The Sympathetic Ganglion. The cytoplasm of the sympathetic ganglion cells adjacent to the adrenal body stained deeply, whereas the nuclei were only slightly stained. The nerve fibres were moderately stained. The satellite cells stained deeply. (Fig. 2).

Comb. The cornified layer was nearly unstained. The epithelium was extremely heavily stained. The lymphoid tissue cells surrounding blood vessels and the fibrous tissue cells were all deeply stained. The blood vessels were most deeply stained. (Fig. 3).

Striated Muscle. The muscle fibres' nuclei stained moderately, the cytoplasm lightly. Connective tissue cells stained deeply in nuclei and lightly in fibres.

Blood/

Blood. The red blood cells stained deeply in the nuclei and with many fine particles in the cytoplasm. The leucocytes stained moderately in the nuclei.

Heart. The cardiac muscle stained moderately in the nuclei, but slightly in the cytoplasm. The pericardium and endocardium stained deeply in the nuclei and lightly in the cytoplasm.

Trachea. The epithelium stained extremely dark in the nuclei. The cartilage was very lightly stained in the nuclei, but not in the ground substance. The ligament between the cartilage rings stained deeply in the nuclei, lightly in the fibres. (Fig. 4).

Lung. The alveolar epithelium stained deeply in the nuclei, lightly in the cytoplasm. The connective tissue was nearly the same. The pleura was only lightly stained in the nuclei. The blood vessels in the lung were just lightly stained in the nuclei of all different layers.

Oesophagus. The inner surface was nearly unstained. The degree of staining increased with the depth of the epithelium and reached its maximum in the germinal layer. The lamina propria was lightly stained. The glandular cells and plain muscle were lightly stained. The connective tissue stained deeply in the nuclei. The serosa was lightly stained.

Proventriculus. The epithelium stained deeply in the nuclei. The connective tissue stained moderately. The basement membrane of the glandular tissue was clearly stained. All the other cells were lightly stained.

Gizzard./

Gizzard. The horny membrane was unevenly and lightly stained. The glandular tissue and the tendinous substance were both deeply stained. The connective tissue and plain muscle only stained deeply in the nuclei. The serosa stained moderately.

Small Intestine. All cells in the villi stained darkly in the nuclei. The glandular cells and connective tissue stained moderately in the nuclei, and lightly in the cytoplasm. The plain muscle stained lightly. The serosa stained unevenly in nuclei. (Fig. 5).

Caecum. All nuclei of the villi and the lymphoid tissue stained deeply. The glandular cells and connective tissue stained moderately in nuclei, lightly in the cytoplasm. The serosa stained lightly. (Fig. 6).

Large Intestine. All nuclei of the mucosa and glandular cells stained deeply, the cytoplasm lightly. All other tissues stained lightly.

Liver. Liver cell nuclei stained intensely, the cytoplasm only lightly. The reticulo-endothelium stained lightly. The connective tissue and lymphoid tissue stained deeply in nuclei, and lightly in cytoplasm. (Fig. 7).

Pancreas. Both the glandular cells and the Langerhan's cells stained deeply in nuclei, lightly in cytoplasm. No detectable difference in enzyme activity was seen in the various islet cells. The connective tissue stained moderately in nuclei. (Fig. 8).

Pineal Body./

Pineal Body. The ependyma stained lightly, and the parenchymal cells stained moderately. The pia mater was deeply stained. (Fig. 9).

Hypophysis. The fibrous capsule was lightly stained in the nuclei, and nearly unstained in cytoplasm. The blood vessels were moderately stained in the nuclei, especially in intima and media, the cytoplasm unstained. (Fig. 10).

Pars distalis. The α cells were rich in acid phosphatase both in nuclei and granules. The β cells were moderately stained, and the chromophobe cells were only lightly stained.

Pars nervosa. The ependymal cells surrounding the sinuses were moderately stained in the nuclei, and lightly in the cytoplasm. The pituicytes were moderately stained in the nuclei only.

Thyroid. All nuclei stained moderately, the cytoplasm slightly, and secretion lightly. (Fig. 11).

Thymus. All thymocytes stained moderately in the nuclei, lightly in the cytoplasm. Both Hassall's bodies and stroma stained moderately. (Fig. 12).

Adrenal Gland. The cortical cells stained deeply in the nuclei, and lightly in the cytoplasm. The medullary cells were more lightly stained. The stroma was moderately stained. (Fig. 2).

Spleen. Those lymphoblasts in the centre of the Malpighian body stained deeper than the surrounding lymphocytes. The stroma was only moderately stained in the nuclei, and the fibres/

fibres were nearly unstained. (Fig. 13).

Kidney. The glomeruli and Bowman's capsules stained deeply in the nuclei and slightly in the cytoplasm. All parts, except the distal convoluted part, of the renal tubules stained deeply in the nuclei, and lightly in cytoplasm. The interstitial tissue was moderately stained in the nuclei only. (Fig. 14).

Bursa Fabricii. The mucosa stained moderately in the nuclei slightly in the cytoplasm. The glandular cells were nearly the same. The lymphoid tissue showed only nuclear staining, with the cytoplasm nearly unstained. Plain muscle and serosa were nearly unstained. (Fig. 15).

Testis. In the testicle of an adult cock the fibrous capsule was moderately stained only in the nuclei. The stroma stained moderately, most of the activity being in the nuclei. The basement membrane stained moderately. All the spermatogonia stained deeply in the nuclei, and lightly in cytoplasm. The head piece of the spermatocyte stained deeply, and the tail lightly.

Ovary. The follicular cells stained deeply in nuclei, and not so intensely in the cytoplasm. The basement membrane stained moderately. The yolk was lightly stained. The interstitial tissue stained lightly. (Fig. 16 and 45).

Oviduct. 1. Infundibulum. The epithelium stained moderately in the nuclei, and lightly in cytoplasm. The submucosa and plain muscle stained lightly in the nuclei, but the cytoplasm was only faintly stained. The serosa was practically unstained.

2. Albumen Secreting Part. The epithelium and lymphoid tissue stained moderately in the nuclei, lighter in the cytoplasm. The glandular cells stained deeply in the nuclei, lightly in the cytoplasm. The plain muscle was lightly stained. The serosa stained very faintly.

3. Shell Forming Part. Only the glandular cells were moderately stained. All other tissues were slightly stained.

(b) In Neoplastic Tissues.

Rous No. 1 Sarcoma. In the actively growing tumour cells the nuclei stained deeply and the cytoplasm was unstained. (Fig. 17). The necrobiotic tumour cells only stained lightly in the nuclei. The stroma was deeply stained in the nuclei and lightly in the cytoplasm. Mitotic figures were comparatively rare in this tumour, but whenever seen they were observed to be clearly stained.

GRCH/15. (dibenzanthracene-induced fibrosarcoma). The tumour cells stained moderately in the nuclei and lightly in the cytoplasm. Numerous mitotic figures stained clearly. The stromal cells were stained more lightly. (Fig. 18).

Campbell Fibrosarcoma. The tumour cells stained moderately in the nuclei and lightly in the cytoplasm. The stroma was lightly stained.

Spontaneous Fibroma. The whole tumour tissue was nearly unstained. Only occasionally fine particles were observed scattered in the nuclei.

Spontaneous Histiocytic Sarcoma. Only faintly stained throughout.

Spontaneous Adenocarcinoma/

Spontaneous Adenocarcinoma. The tumour cells stained moderately in the nuclei and lightly in the cytoplasm. The stroma was nearly unstained. (Fig. 19).

Spontaneous Lymphocytoma. The tumour cells stained deeply in nuclei and lightly in the cytoplasm. The stroma stained moderately in the nuclei and was nearly unstained in the cytoplasm. (Fig. 20).

Spontaneous Fibroleiomyoma. ("Fibroid" of the oviduct ligament). All nuclei stained deeply, but the cytoplasm was unstained. (Fig. 21).

(2) The Distribution of Alkaline Phosphatase.

(a) In the Tissues of Normal Chickens.

The word "stained" used in the following descriptions means the location of the black cobalt sulphide precipitate at the site of alkaline phosphatase activity.

Central Nervous System. All nerve fibres stained moderately. Microglia cells stained most deeply in the nuclei, followed by the ependyma, chorioidea, oligodendroglia, Pukinje cells, neurons, astrocytes, and pia mater. The blood capillaries stained very deeply.

The Sympathetic Ganglion. In the suprarenal ganglion, all nerve fibres stained deeply, but the neurons stained lightly in nuclei and cytoplasm. (Fig. 22).

Comb. The cornified layer was unstained. The degree of nuclear staining increased gradually in accordance with the depth/

depth of the epithelium and reached the maximum in the Malpighian layer. The cytoplasm however, was only lightly stained. The papillary layer and lymphoid cells stained moderately in the nuclei and lighter in the cytoplasm. The connective tissue stained deeply in the nuclei, and moderately in the fibres. The blood capillaries were very darkly stained.

Striated Muscle. Muscle fibres stained moderately in the nuclei and lightly in the cytoplasm. The connective tissue stained deeply in the nuclei and lightly in the cytoplasm.

Blood. The red blood cells stained moderately in the nuclei and lightly in the cytoplasm. Leucocytes stained deeply in the nuclei and lightly in the cytoplasm.

Heart. Both the pericardium and the cardiac muscle showed deep nuclear, and light cytoplasmic staining. The endocardium stained extremely deeply in nuclei and cytoplasm.

Blood Vessels. The endothelium was intensely stained in the nuclei, and more light in the cytoplasm. The media and adventitia stained deeply in the nuclei and moderately in the cytoplasm. Blood capillaries stained deeply in the nuclei and moderately in the cytoplasm.

Trachea. The fibro-elastic layer stained moderately in the nuclei and lightly in the fibres. All other cells stained deeply in the nuclei and lightly in the cytoplasm. (Fig. 23).

Lung. The alveolar epithelium and interstitial tissue were both deeply stained in the nuclei and lightly in the cytoplasm. The pleura stained moderately in the nuclei and more lightly in the cytoplasm. (Fig. 24).

Oesophagus./

Oesophagus. In the deeper part of the stratified squamous epithelium nuclear staining increased to the highest degree in the germinal layer. The lamina propria stained deeply in the nuclei and lightly in the cytoplasm. The mucous gland and the plain muscle stained moderately in the nuclei and lighter in the cytoplasm. The connective tissue stained deeply in the nuclei and lightly in the fibres. The serosa stained moderately. (Fig. 25).

Proventriculus. The epithelium stained moderately in the nuclei and more lightly in the cytoplasm. The glandular cells stained moderately and the basement membrane deeply. Plain muscle stained moderately, and the serosa stained lightly. (Fig. 26).

Gizzard. The horny lining stained lightly. The glandular tissue stained deeply and the cytoplasm lightly. The connective tissue stained moderately in the nuclei and lightly in the cytoplasm. The plain muscle stained lightly. The tendinous substance stained deeply in the nuclei and lighter in the cytoplasm. The serosa stained lightly.

The Small Intestine. The villi stained deeply in the nuclei and on the free surface. The glandular cells and connective tissue stained moderately in the nuclei and lighter in the cytoplasm. The plain muscle stained lightly, and the serosa stained moderately. (Fig. 27).

Caecum. The mucosa and lymphoid tissue stained deeply in the nuclei and more faintly in cytoplasm. The glandular cells stained moderately. Plain muscle stained lightly. Connective/

Connective tissue and serosa stained moderately in the nuclei and lighter in the cytoplasm. (Fig. 28).

Large Intestine. The mucosa and glandular cells stained moderately in the nuclei and more lightly in the cytoplasm. Plain muscle stained lightly. Connective tissue stained deeply in the nuclei and lighter in the cytoplasm. The serosa was moderately stained.

Liver. The reticulo-endothelium stained deeply and the network of reticulin fibrils showed up very clearly. All the other cells stained moderately in the nuclei and lighter in the cytoplasm. (Fig. 29).

Pancreas. The glandular cells stained moderately in the nuclei and lightly in the cytoplasm. The Langerhan's cells and the interstitial tissue stained a little deeper. (Fig. 30).

Pineal Body. The ependyma stained extremely deeply. Again no distinction could be made between the different types of islet cell. The parenchyma stained moderately. The pia mater stained deeper in cytoplasm. (Fig. 31).

Hypophysis. The fibrous capsule was moderately stained in the fibres, and a little deeper in the nuclei. The blood vessels were moderately stained only in the intima and surrounding areolar tissue. (Fig. 41). Nerve fibres stained moderately. Connective tissue showed very deep staining both in nuclei and fibres. (Fig. 32).

Pars distalis./

Pars distalis. All the three different kinds of cells were moderately stained in the nuclei and lightly in the cytoplasm.

Pars nervosa. The ependymal cells and the pituicytes were all lightly stained both in nuclei and cytoplasm.

Thyroid. The glandular cells stained deeply in the nuclei and lightly in the cytoplasm. The secretion was lightly stained. Connective tissue stained moderately in the nuclei and lighter in the fibres. (Fig. 33).

Thymus. Thymocytes and Hassall's bodies stained moderately in the nuclei and slightly in the cytoplasm. The stroma stained lightly. (Fig. 33).

Adrenal Gland. The cortical cells stained deeply in the nuclei and lightly in the cytoplasm. The medullary cells stained moderately in the nuclei and lightly in the cytoplasm. The stroma stained moderately. (Fig. 22).

Spleen. The lymphoblasts in the centre of the white pulp stained deeper than the surrounding area. The connective tissue showed moderate staining in the nuclei and lighter staining in the cytoplasm. (Fig. 34).

Kidney. The proximal convoluted tubules stained deeper in cytoplasm than other parts of the renal tubules, and the brush border was especially deeply stained. The connective tissue was also stained more deeply in fibres. All the other cells stained moderately in the nuclei and more lightly in the cytoplasm. (Fig. 35).

Bursa Fabricii./

Bursa Fabricii. The epithelial and glandular cells all stained moderately in the nuclei and lighter in the cytoplasm. The lymphoid tissue and plain muscle stained lightly. The serosa stained moderately. (Fig. 36).

Testis. Both the fibrous capsule and the stroma were deeply stained. The Sertoli cells were stained moderately in nuclei and lightly in cytoplasm. The spermatogonia stained rather deeply in the nuclei. The heads of spermatids stained moderately, and the tails lightly.

Vas deferens. The nuclei of the epithelium were moderately stained, and the cytoplasm was unstained. Connective tissue stained moderately.

Ovary. The follicular cells stained moderately in the nuclei, lighter in the cytoplasm. Yolk stained lightly. The basement membrane stained clearly, and connective tissue stained moderately. (Fig. 46).

Oviduct. 1. Infundibulum. The brush border stained moderately. The epithelial cells stained lightly in the nuclei, the nucleoli were clearly stained, and the cytoplasm deeply stained. The submucosa was nearly unstained. The plain muscle stained lightly in the nuclei, lighter in the cytoplasm. The serosa was nearly unstained.

2. Albumen Secreting Part. The epithelium stained lightly in nuclei, and even more lightly in the cytoplasm. The coarse secretion granules in the cytoplasm of the glandular cells were slightly stained. The nuclei of the lymphoid cells stained lightly, and the cytoplasm was nearly unstained. The plain/

plain muscle was also nearly unstained. The serosa stained lightly.

3. Shell Forming Part. The epithelial cells were only lightly stained in the nucleoli. The coarse cytoplasmic granules of the glandular cells stained lightly. Plain muscle and serosa were nearly unstained.

(b) In Neoplastic Tissues.

Rous No. 1 Sarcoma. The nuclei of the tumour cells showed a variation of light to deep staining and the cytoplasm was nearly unstained. Staining was more deep where active growth was going on, and was almost absent in necrobiotic areas. The location of the enzyme activity in the nuclei was chiefly limited to the nucleoli and the nuclear membrane. The stroma was stained deeply in the nuclei, and very lightly in the fibres. (Fig. 37).

Campbell Fibrosarcoma. Most cells were practically unstained. Only a few parenchymatous cells showed moderate staining.

GRCH/15 (dibenzanthracene induced fibrosarcoma). This tumour does not appear to be rich in alkaline phosphatase. Tumour cells stained moderately in the nuclei and lighter in the cytoplasm. The stroma stained lightly. (Fig. 38).

Spontaneous fibroma. Only the nucleoli of the tumour cells stained lightly. Nuclei of the stroma stained slightly.

Spontaneous Histiocytic Sarcoma./

Spontaneous Histiocytic Sarcoma. Only the nucleoli of the tumour cells stained lightly, and the cytoplasm was nearly unstained. The fibrous capsule stained deeply. The endothelial cells of the blood vessels, the nuclei of the media, and the lymphoid cells near the blood vessels all stained deeply. (Fig. 44).

Spontaneous Adenocarcinoma. Tumour cells stained deeply in the nuclei, and lightly in the cytoplasm. The stroma deeply stained both in nuclei and fibres. (Fig. 42).

Spontaneous Lymphocytoma. Tumour cells stained moderately in nuclei and more lightly in the cytoplasm. The stroma only stained moderately in the nuclei.

Spontaneous Fibroleiomyoma. The tumour cells stained lightly in the nuclei and even more lightly in the cytoplasm. The stroma stained moderately in the nuclei and fibres. (Fig. 43).

Enzyme Activity.

Key: - None
± Trace
\+ Moderate
++ Fairly Active
+++ Active
++++ Very Active

An arbitary system of plus and minus signs has been adopted to indicate enzyme activity.

TABLE I./

TABLE I.

The Histochemical Distribution of Acid and Alkaline Phosphatase in the Cells of the Central Nervous System.

Cell.	Phosphatase	Nucleolus	Nuclear Membrane	Neuro-fibrile	Axon	Dendrite
Neuron	Acid	+	++	+++	+++	+
	Alkaline	++	+	+	++	++
Purkinje cell	Acid	+	±	±	-	-
	Alkaline	+++	+	±	+	+
Oligodendroglia	Acid	+++	+++	±	±	±
	Alkaline	++	++	±	+	+
Astrocyte	Acid	+	++	-	-	-
	Alkaline	+	++	±	+	+
Microglia	Acid	++	+++	+	+	+
	Alkaline	+++	+++	±	+	+
Chorioidea	Acid	+	+			
	Alkaline	++	+++			
Ependyma	Acid	+++	+			
	Alkaline	+++	+++			
Pia Mater	Acid	++	+			
	Alkaline	+++	++			
Blood Capillary	Acid	+	±			
	Alkaline	++	+++			

TABLE II.

The Histochemical Distribution of Acid and Alkaline Phosphatases in Healthy 6-week-old Chicken Tissues.

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Comb	Cornified layer		±		±
	Stratum lucidum		±		±
	Stratum granulosum	++	+++	+	±
	Stratum Malpighii	+++	++++	+++	+
	Papillary layer of derma	+++	+	++	+
	Lymphoid cells	+++	++	++	+
	Fibrous tissue	+++	++	+++	++
	Blood capillaries	+++	+++	+++	+++
Muscle	Striated Muscle	++	+	++	+
	Interstitial tissue	+++	+	+++	+
Blood	r.b.c.	+++	+	++	+
	w.b.c.	++	±	+++	+
Heart	Pericardium	+++	+	+++	+
	Myocardium	++	±	+++	+
	Endocardium	+++	±	++++	+++
Artery	Endothelium	±	-	++++	+++
	Media	±	-	+++	+
	Adventitia	±	-	+++	++

Vein/

TABLE II. (continued).

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Vein	Endothelium	±	-	++++	+++
	Media	±	-	++	++
	Adventitia	±	-	+++	++
Capillary		++	±	+++	++
Trachea	Mucosa	++++	±	+++	+
	Cartilage	±	-	+++	+
	Ligament	+++	+	+++	+
	Fibro-elastic layer	+++	±	++	+
Lung	Alveolar epithelium	+++	±	+++	+
	Interstitial tissue	+++	+	+++	+
	Pleura	+	-	++	+
Oeso-phagus	Epithelium	±	-	+	±
	Germinal layer	+++	±	+++	++
	Lamina propria	±	±	+++	+
	Muscularis mucosae	++	±	++	+
	Mucous gland	+	±	++	+
	Plain muscle	+	±	++	+
	Connective tissue	+++	+	+++	+
	Serosa	+	±	++	+

TABLE II. (continued).

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Proventriculum	Epithelium	+++	±	++	±
	Connective tissue	++	±	+++	++
	Glandular tissue	±	±	++	+
	Plain muscle	±	±	++	+
	Serosa	±	±	+	±
Gizzard	Horny lining		+		+
	Glandular tissue	+++	++	+++	+
	Connective tissue	+++	±	++	±
	Plain muscle	+++	+	±	±
	Tendinous substance	+++	++	+++	++
	Mesothelium	++	+	+	±
Duodenum	Mucosa	+++	±	+++	+
	Glandular cells	++	+	++	+
	Plain muscle	±	±	+	±
	Connective tissue	±	±	++	±
	Serosa	±	±	+	±
Jejunum	Mucosa	+++	+	+++	++
	Glandular cells	+	+	++	+
	Plain muscle	+	±	+	±
	Connective tissue	+++	+	++	+
	Serosa	++	±	++	+

Ileum/

TABLE II. (continued).

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Ileum	Mucosa	+++	±	+++	+
	Glandular cells	++	±	++	+
	Plain muscle	+	±	+	±
	Connective tissue	++	±	+	±
	Serosa	++	±	++	+
Caecum	Mucosa	+++	±	+++	+
	Lymphoid tissue	+++	±	+++	+
	Glandular cells	++	+	++	+
	Plain muscle	+	±	+	±
	Connective tissue	++	±	++	+
	Serosa	+	±	++	+
Colon	Mucosa	+++	±	++	+
	Glandular cells	+++	±	++	+
	Plain muscle	±	±	+	±
	Connective tissue	+	±	+++	++
	Serosa	+	±	++	+
Liver	Liver cell	+++	±	++	+
	Sinusoid endothelium	++	+	++	++
	Bile duct	++	±	++	+
	Connective tissue	+++	±	++	+
	Lymphoid cells (present group normally in birds.)	+++	±	++	+

Pancreas/

TABLE II. (continued).

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Pancreas	Glandular cells	+++	+	++	±
	Interstitial tissue	++	±	++	+
	Langerhan's cells	+++	+	++	+
Pineal body	Ependyma	+	+	+++	+++
	Parenchymal cells	++	+	++	+
	Pia mater	+++	++	++	+++
Hypo-physis	Fibrous capsule	±	-	++	+
Pars distalis	α-chromophile	++	++	++	+
	β-chromophile	++	+	++	+
	Chromophobe	+	±	++	+
Pars nervosa	Ependymal cells	++	+	+	+
	Pituicytes	++	±	+	+
Thyroid	Glandular cells	++	±	+++	+
	Secretion		±		+
	Interstitial tissue	++	±	++	+
Thymus	Thymocytes	++	±	++	±
	Hassall's body	+	±	++	±
	Stroma	+	±	+	±
Adrenal gland	Cortical cells	+++	±	+++	+
	Medullary cells	++	+	++	+
	Stroma	++	+	++	++
Spleen/					

TABLE II. (continued).

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Spleen	Malpighian body	+++	±	+++	+
	Red blood cells	+++	+	++	±
	Trabeculi	++	±	++	+
	Capsule	++	±	++	+
Kidney	Glomerulus	+++	±	++	+
	Bowman's capsule	+++	±	++	+
	Proximal convoluted tubule	+++	+	++	++
	Descending limb of Henle's loop	+++	+	++	+
	Ascending limb of Henle's loop	+++	+	++	+
	Distal convoluted tubule	++	±	++	+
	Collecting tubule	+++	+	++	+
	Interstitial tissue	++	±	++	++
Bursa Fabricii	Mucosa	++	±	++	+
	Lymphoid tissue	+	±	+	±
	Glandular cells	++	±	++	±
	Plain muscle	±	±	+	±
	Serosa	±	±	++	+
Testis	Germinal cells	+++	+	+++	+
	Stroma	++	+	+++	++

Ovary/

TABLE II. (continued).

Organ.	Tissue	Acid Phosphatase Nucleus	Acid Phosphatase Cytoplasm	Alkaline Phosphatase Nucleus	Alkaline Phosphatase Cytoplasm
Ovary	Yolk		±		+
	Follicular cells	+++	+	++	+
	Basement membrane	++	±	++	++
	Interstitial tissue	+	±	++	++
Oviduct Infundibulum	Epithelium	++	+	++	+
	Submucosa	+	±	±	±
	Plain muscle	+	±	+	±
	Serosa	+	±	+	±
Albumen secreting part	Epithelium	++	+	+	+
	Glandular cells	+++	+	±	+
	Lymphoid cells	++	+	+	±
	Plain muscle	+	±	±	-
	Serosa	±	±	+	+
Shell forming part	Epithelium	+	+	+	-
	Glandular cells	++	+	±	+
	Plain muscle	±	±	±	±
	Serosa	±	±	±	±

TABLE III

The Histochemical Distribution of Acid and Alkaline Phosphatases in Neoplastic Chicken Tissues.

Type of Tumour	Tissue	Acid Phosphatase		Alkaline Phosphatase	
		Nucleus	Cytoplasm	Nucleus	Cytoplasm
Rous No. 1 Sarcoma	Tumour cells				
	(a) living	+++	-	+++	±
	(b) necrobiotic	+	-	+	-
	Stroma	+++	+	+++	±
GRCH/15 Sarcoma	Tumour cells	++	±	++	++
	Stroma	++	±	++	+
Spontaneous Fibroma	Tumour cells	±	±	+	±
	Stroma	±	±	+	±
Spontaneous Histiocytic Sarcoma	Tumour Cells	±	±	±	±
	Stroma	±	±	±	±
Spontaneous Adenocarcinoma	Tumour cells	++	+	+++	+
	Stroma	+	±	+++	++
Spontaneous Lymphocytoma	Tumour cells	+++	+	++	+
	Stroma	++	±	++	+
Spontaneous Fibroleiomyoma	Tumour cells	+++	-	+	±
	Stroma	+++	-	++	++
Campbell Fibrosarcoma	Tumour cells	++	±	±	±
	Stroma	+	±	±	±

B. Biochemical Study.

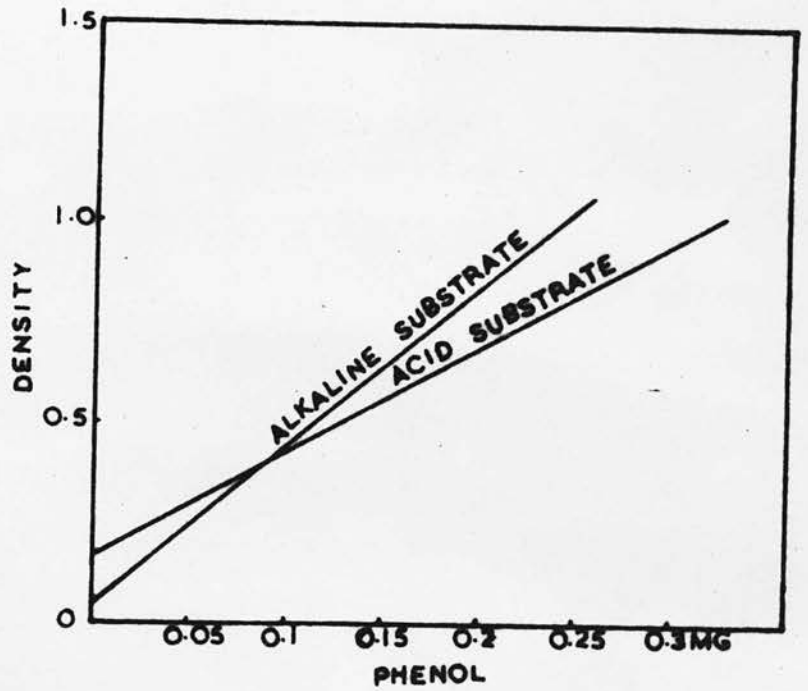
The biochemical quantitative method used in the present work was chiefly based upon Kay-Graham's 'long test' for the estimation of alkaline phosphatase in milk (Elsdon and Walker, 1942), and Gutman and Gutman's method (1940) for the estimation of acid phosphatase in serum. Densities of the coloured solutions were read with a Hilger "Spekker" Absorptiometer, using Ilford Spectrum yellow (No. 606) and Chance's "Calorex" heat absorbing filters. The graph relating density to phenol was found to be two straight lines over the range used for both the acid and alkaline substrate solutions. (See next page).

For each sample of fresh tissue to be tested, 0.2 gm. was accurately weighed out, ground together with a little chemically pure quartz sand (No. 6089, A. L. Curtis Westmor Laboratory, Chatteris) in a glass mortar, 10 ml of distilled water was added and allowed to stand for half an hour before filtering through Whatman No. 1 filter paper, 0.5 ml of this filtrate (1:50) was diluted with 19.5 ml distilled water in order to make it up to 1:2000 dilution, and finally 2 ml of this diluted tissue extract was added to 10 ml of substrate solution just before incubation. The remaining tissue extract was kept in the refrigerator until next day and 2 ml was added to the control tubes after incubation and cooling.

For the estimation of acid phosphatase, the substrate was prepared as follows:

Solution A. Dissolve 1.092 gm. of disodium phenyl phosphate in 500 ml distilled water. (0.005 M.)

Solution B./



GRAPH SHOWING CO-RELATION BETWEEN DENSITY AND INCREASING AMOUNT OF STANDARD PHENOL SOLUTION.

Solution B. Dissolve 42.0 gm. of crystaline citric acid in distilled water, add 376 ml of 1 N. sodium hydroxide, make up to 1 litre. Preserve in well stoppered bottles in a refrigerator. The pH was checked to 5.0 with the aid of bromo-cresol green standard colour tubes. Equal parts of solution A and B were mixed just before use.

The substrate solution used for the estimation of alkaline phosphatase was prepared in the same way except that barbiturate buffer was used (pH 9.4) instead of the fore-mentioned 0.1 M citrate buffer (pH 5.0). 11.54 gms. of sodium barbitone were dissolved in 500 ml distilled water and kept in well stoppered bottles in the refrigerator. An equal amount of disodium phenyl phosphate solution was added to the above buffer just before use.

The general procedure for estimating phosphatases was as follows:-

(1) To each 10 ml of substrate solution 3 drops of chloroform and 2 ml of tissue extract (1:2000) were added and placed in the incubator at 37°C. together with a duplicate and a control without tissue extract, for exactly 22 hours.

(2) After incubation, the tubes were cooled in the refrigerator for 20 minutes, 2 ml of tissue extract was added to the control tube, and then 4.5 ml of diluted Folin-Ciocalteu's reagent (1:3 in distilled water just before use) was added to each tube.

(3)/

(3) After mixing well, 10 ml was taken from each tube, and 2 ml of a 14% anhydrous sodium carbonate solution was added to each tube.

(4) The tubes were plunged immediately into a bath of boiling water for exactly 2 minutes.

(5) They were then cooled in running cold water and read against distilled water with the Absorptiometer.

(6) The amount of phenol released from disodium phenyl phosphate by enzyme activity was then calculated from the standard calibration curve.

For example,

0.1 mg phenol gave a density in acid substrate solution of 0.256.

2 ml 1:2000 normal chick's liver extract gave a density in acid substrate solution after 22 hours incubation of 0.708.

This corresponds to $\frac{0.1 \times 0.708}{0.256} = 0.2765$ mg phenol

Therefore 276.5 mg of phenol will be released through the activity of acid phosphatase contained in 1 gm of fresh liver after 22 hours incubation at 37°C.

The quantitative results for different kinds of normal and neoplastic tissues were condensed in the following tables:

TABLE IV./

TABLE IV.

Acid and Alkaline Phosphatase Activity in 18 Different Organs of Normal 6-week-old Chicks.

Organ	Acid Phosphatase (mg. phenol released by 1 gm of tissue after 22 hours incubation at 37°C.)				Alkaline Phosphatase. (mg. phenol released by 1 gm. of tissue after 22 hours incubation at 37°C.)			
Cerebrum	80.5				19.37			
Cerebellum	138.6				15.3			
Pituitary	170.2			54.3	191.2			67.7
Thyroid	120.3				12.2			
Parathyroid	47.96				44.96			
Thymus	189.4	110.9				5.26		
Pancreas	207.8				8.1			
Adrenal	98.4				18.6			
Heart	94.9				98.0			
Lung	87.5	42.5	46.5	52.3	11.0	11.24	7.65	
Spleen	215.0	337.1	215.6	299.6		7.65	6.45	
Bone marrow	109.7	100.0	111.3	90.2	20.8	7.4	11.8	7.6
Kidney	94.1	201.9	107.4	163.6	97.1	187.5	114.5	80.8
Testis	125.7				68.9			
Proventriculum	112.1				35.4			
Duodenum	257.8				92.1			
Liver	276.5	273.8	185.9	317.9	102.2	126.07	45.4	66.7
Muscle	85.9		17.5	22.6	8.3			11.0

TABLE V.

Acid and Alkaline Phosphatase Activity in Various Tissues of GRCH/15 Tumour-bearing Chicks.

Tissue	Acid Phosphatase. (mg phenol released by 1 gm. of tissue after 22 hours incubation at 37°C.)			Alkaline Phosphatase. (mg. phenol released by 1 gm. of tissue after 22 hours incubation at 37°C.)		
			Average			Average
Tumour	178.0	76.6	127.3	14.2	15.8	15.0
Muscle	90.6	98.8	94.7	0.109	4.1	2.104
Liver	198.0	228.9	213.4	9.8	24.4	17.1
Lung	122.0	101.9	111.9	4.5	18.4	11.4
Spleen	383.2	310.5	341.8	3.5	5.5	4.5
Kidney	205.8			40.4		
Bone marrow		121.1			3.5	

TABLE VI.

Acid and Alkaline Phosphatase Activity in Various Tissues of Rous Tumour-bearing Chicks.

Tissue	Acid Phosphatase (mg. phenol released by 1 gm. of tissue after 22 hours incubation at 37°C.)			Alkaline Phosphatase. (mg. phenol released by 1 gm. of tissue after 22 hours incubation at 37°C.)		
Tumour	9.75	24.6		1.55	0	
Muscle	27.5	41.4		6.3	3.3	1.91
Liver	293.6	332.4	116.4	96.5	83.7	10.47
Lung	59.5	72.6		22.6	2.8	
Kidney	128.8	178.1	139.8	79.3	123.6	105.02
Bone marrow	65	159.3	46.8	15.4	21.3	4.06

IV. Discussion.

In any work on enzymes, whether biochemical or histochemical, the problem of artefacts must be given consideration. There have been lengthy discussions on the possibility of enzyme diffusion rendering the interpretation of histochemical findings of doubtful value, and various methods for combatting this have been suggested. It is difficult however, to conceive diffusion taking place to any extent in the Gomori technique and its modifications, as the initial treatment, by coagulating the proteins of the tissue, would tend to confine the enzyme to its original site at the moment of fixation, and none of the subsequent steps in processing leading up to embedding in paraffin, are favourable for enzyme diffusion.

It has been suggested that the process of de-paraffinization and taking the sections down to water preparatory to placing in the substrate, might result in the diffusion of soluble enzymes, and a method has been described wherein the section, still impregnated with paraffin wax, is placed in the substrate (Ruyter and Neumann, 1949). In the writer's hands however, this method has not given good results. This does not seem surprising, as the enzyme and substrate can hardly come into intimate contact so that hydrolysis can take place. It is now generally accepted that Gomori's technique is reliable (Danielli, 1946), and indeed, the sharpness of definition of a good histochemical preparation for acid or alkaline phosphatase seems good evidence that diffusion can hardly influence the results.

It was noticed however, that the histochemical picture was sometimes patchy when the usual method of incubating sections on slides placed vertically in a jar of substrate was followed. The greatest activity was often seen to be in that part of the section nearest the surface of the substrate; and it was conceived possible that oxygen tension might influence the speed of the reaction. The method was therefore modified, in that sections were mounted on cover-slips and incubated horizontally in a Petri dish of substrate. In this manner, every part of the section was an equal depth below the interphase, and it was found that the results were much improved.

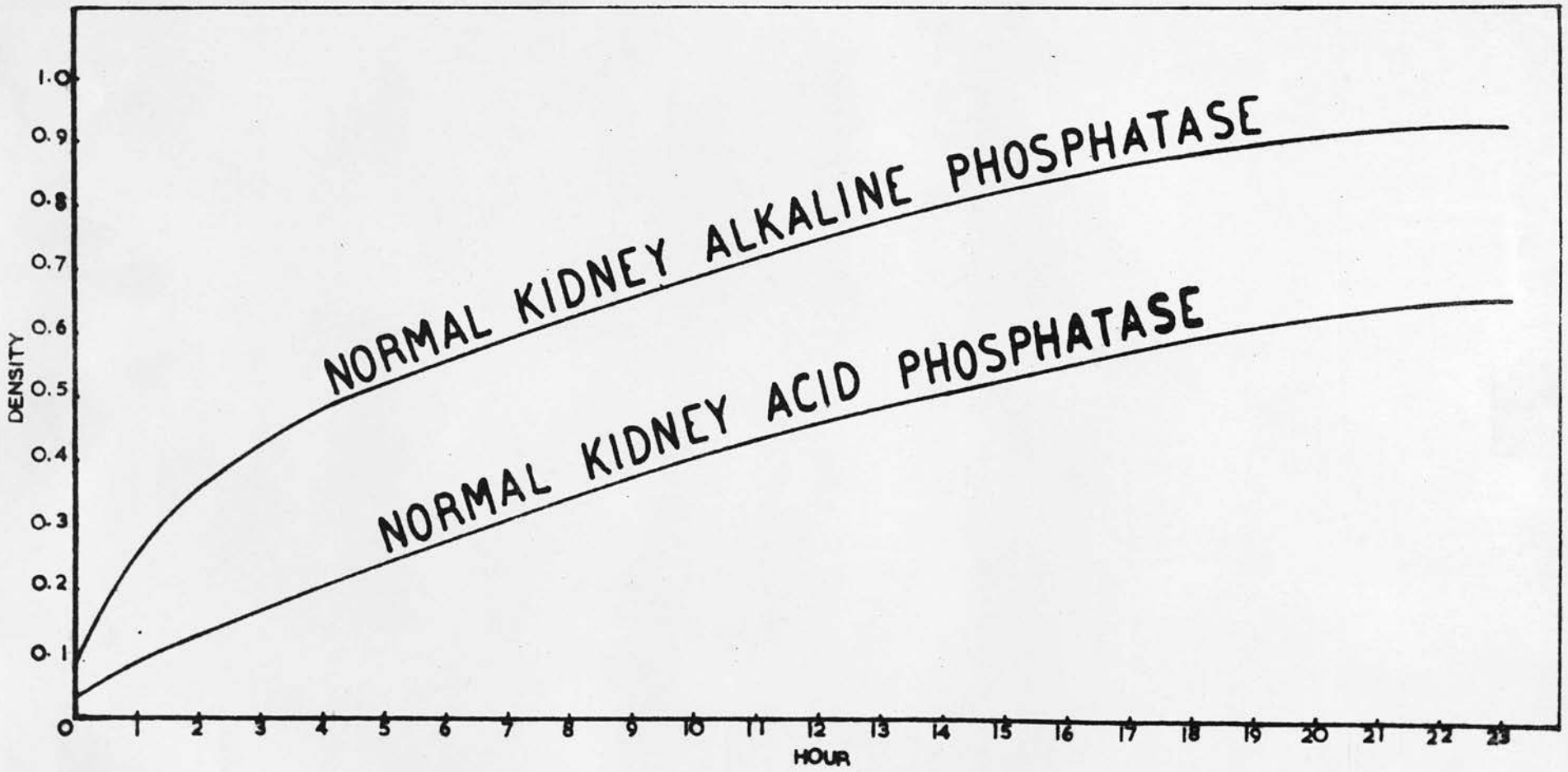
The question of stability of acid and alkaline phosphatases then had to be considered. Compared with many other enzymes, the phosphomonoesterases are remarkably stable, but even so, it was found that acid phosphatase is more stable in aqueous extract than alkaline phosphatase. It is thought that the instability of the latter may be due to the splitting down of the enzyme into its component parts i.e. the co-enzyme and its carrier or apo-phosphatase (Albers et al 1948). It has been found that there is no pH change of tissue extract after storage in the refrigerator and in the absence of bacterial contamination, even after a fortnight. As all quantitative work was done as rapidly as possible and under standard conditions and time, the question of artefact due to degradation of enzyme activity need not be considered.

The/

The co-relation between the density of the substrate fluid and the time of incubation has been tested by putting twenty-four duplicates into the incubator at 37°C. and then taking one set every hour to measure the density with an absorptiometer. The result for kidney phosphatases is illustrated in the graph on the following page. Generally speaking, after 22 hours incubation the density remains constant both for acid and alkaline phosphatase.

Very little is known regarding the function of the phosphomonoesterases. They hydrolyse the monoesters of the phosphoric acid, and the alkaline enzyme, also known as blood plasma or bone phosphatase, is identical with nucleotidase, splitting off phosphate from nucleotides. A great deal of hypothetical discussion of the functions of these enzymes has taken place, most of which is mentioned in the introduction. It is certain that much remains to be discovered about the physiological function of the phosphomonoesterases, and any histochemical or biochemical work which throws light on this must be of some value.

In this series of experiments, whenever possible, histochemical observations were checked by quantitative studies, and in no case were any major discrepancies found. An interesting feature emerged which appears to lend support to the view that both acid and alkaline phosphatase are involved in growth and differentiation. This was that regions where cells were actively proliferating such as the Malpighian layer of the epidermis, (Fig. 3) the basal layer of the oesophageal epithelium/



GRAPH SHOWING CO-RELATION BETWEEN DENSITY AND TIME OF INCUBATION.

epithelium (Fig. 25), and in the intestinal mucosa (Fig. 5, 6, 27 and 28), were found to be rich in enzyme. Primitive Graafian follicles are poor in acid phosphatase activity, but once they commence to mature, a greatly increased enzyme activity becomes manifest (Fig. 45). On the other hand, there is no such difference in the distribution of alkaline phosphatase (Fig. 46). It is noteworthy that in rapidly growing tumours, the chromosomes of dividing cells are deeply stained with the method for acid phosphatase (Figs. 17 and 18). This phenomenon is not however confined to neoplastic conditions, since it is also observed in the epithelium of the intestine.

Other organs rich in enzyme are the oviduct — especially the shell secreting part which has a high alkaline phosphatase activity; the kidney where the renal tubules are deeply stained, especially at the brush borders, by the method for alkaline phosphatase (Fig. 35); and the brain, where some motor neurones and especially their associated neurofibrils, had a high acid phosphatase activity (Fig. 1) whilst adjacent neurones were only lightly stained. It is known that all neurones are not in a simultaneous state of activity and the above finding appears to indicate that acid phosphatase reflects cellular activity in nerve cells.

Certain discrepancies between the present results and the findings of other authors have been noticed, and must be briefly mentioned.

According/

According to Yao (1949): "During Drosophila embryogenesis, acid phosphatase shows no change of activity, at least cytochemically; whereas alkaline phosphatase arises only after the contraction of the germ band, increases in activity and then decreases again." In chick embryogenesis, according to Moog (1944, 1946), the situation is slightly different because the concentration of alkaline phosphatase is much greater than that of acid phosphatase in the early stages of development; nevertheless, the extent of the change of acid phosphatase activity between the 2 and 12 days old chick embryo is far less marked than that of alkaline phosphatase in the same period. Also according to Lindeman (1949), the acid phosphatase activity of the embryonic chick retina is constant compared with the alkaline phosphatase from the twelfth day of incubation until 100 days after hatching. It is apparent that these findings are not in accord with the present observations. This discrepancy might be due to some difference in enzyme functions between embryogenesis and oogenesis. Further quantitative studies should be undertaken to clarify this point.

Wolf, Kabat and Newman (1943) state there is less acid phosphatase activity in the Malpighian bodies of the spleen compared with that of the red pulp, and there is no acid phosphatase at all in the stratified epithelium of the oesophagus. According to the present work however, there is no evident difference between the Malpighian bodies and the red pulp of the spleen, and the germinal layer of the epithelium/

epithelium of the oesophagus is fairly rich in acid phosphatase. This might be accounted for by some fundamental difference in the chemistry of these cells between mammals and birds.

According to Kabat and Furth (1941): "The epithelial cells of the large intestine (of certain mammals and the chicken) did not give the (alkaline phosphatase) reaction except a few cells in close proximity to the lumen." The present observations however show the epithelium of the colon of the chicken to be rather deeply stained (Fig. 28).

In the same paper they say: "The bile ducts and liver cells contained no phosphatase, or only traces, although only occasionally fine granules were seen scattered throughout the section but not identified with any cell type." However, the writer found a moderate amount of alkaline phosphatase both in the bile ducts and the liver cells of the chicken (Fig. 29).

The same report states that: "Thyroid epithelium from a young (human) adult did not contain phosphatase." In the chicken however the epithelial nuclei of the thyroid are fairly rich in alkaline phosphatase whereas the cytoplasm is less active (Fig. 33).

In general it has been found that alkaline phosphatase is present in the connective tissue stroma or basement membrane of organs, whilst acid phosphatase is mainly present in the nuclei. It is therefore curious that in the livers of GRCH/15 sarcoma bearing chickens, which have been found to be frequently affected with a fine diffuse intralobular cirrhosis, there is a pronounced decrease in alkaline phosphatase activity compared with/

with normal liver. (Average for normal liver 85.0 units and for liver of GRCH/15 birds 17.1 units See Tables 4 and 5). Liver damage might be expected to ensue from the presence of a large rapidly growing tumour in the breast, due to toxic products resulting from breakdown and necrotic changes. The GRCH/15 sarcoma however is not so susceptible to haemorrhage and necrosis as the Rous I sarcoma, and yet the livers of birds bearing the latter are not affected in this manner, in fact the enzyme activity shows a slight though not necessarily significant increase of alkaline phosphatase as compared with the liver from normal chickens (102.7 units compared with 85.0 in normal bird). GRCH/15 sarcoma is a chemically induced tumour (dibenzanthracene), but since it was originally induced in 1935 by Dr. P. R. Peacock it has been transplanted so many times through so many generations of chickens that the question of liver damage by the original inducing agent cannot be entertained.

The Rous I sarcoma is associated with an infective agent or "virus" and thus presumably differs fundamentally in its metabolic processes from the GRCH/15 tumour. It must be assumed that the toxins liberated from necrotic tissue have little to do with the liver damage, which may be due to some other metabolic product of the tumour cell specific to GRCH/15 sarcoma.

It/

It has been shown that various hepatic disorders give rise to an increased serum alkaline phosphatase. In rat hepatoma, the enzyme is also present in the tumour in increased amount (Greenstein 1947 p. 219). This phenomenon has been explained by assuming that alkaline phosphatase is derived from bone forming cells, and is normally excreted via the bile. Liver derangement then results in faulty excretion followed by a rise in concentration of the enzyme in the serum. If this view is correct, it is difficult to account for the low alkaline phosphatase content of the damaged liver in GRCH/15 bearing birds. Unfortunately the serum phosphatase level was not investigated, but judging from the levels found in other organs of these tumour bearing birds, no very significant rise in enzyme activity would have been detected.

The normal liver of the young chicken is usually dark brown in colour, soft and friable, and contains very little connective tissue between the liver cords. (Fig. 47). In comparison the cirrhotic livers of those birds bearing the GRCH/15 tumour are purplish in colour, firm and enlarged and contain a substantially increased amount of connective tissue dividing the liver cells into numerous small groups (Fig. 48). Higher magnification shows the liver cells of these cirrhotic livers to have a marked variation in nuclear size. Many newly formed bile ducts appear amongst the hypertrophied connective tissue. All the central veins and the blood sinusoids are congested. Yellowish, serous ascites often accompanies this diffusive/

diffusive cirrhotic change. Alkaline phosphatase in histochemical preparations of the cirrhotic liver containing metastases of GRCH/15 sarcoma, is limited to an area surrounding the central vein of some liver lobules (Fig. 52). In the normal liver the enzyme appears to be evenly distributed throughout the organ (Fig. 51).

Acid phosphatase in the normal liver is visible histochemically in moderate amount in every nucleus (Fig. 49). In the cirrhotic liver of birds carrying the GRCH/15 tumour there is a decrease of acid phosphatase activity in the compression zone at the periphery of the metastatic tumour (Fig. 50).

Since alkaline phosphatase plays an important role in phosphorylation, and the liver is the most important organ undertaking this function, it is probable that the process must be seriously impeded in the cirrhotic liver.

In birds carrying the Rous I sarcoma, the livers are histologically normal, and the alkaline phosphatase activity is substantially similar to that of the livers of normal birds.

The marked variation between the two alkaline phosphatase assays for the pituitary of the normal chicken indicates a possible interesting sideline to this investigation which might be followed up by more intensive study when time allows.

V. Summary and Conclusions./

V. Summary and Conclusions

1. Eighteen different tissues from normal 6-week-old chickens and various tissues from tumour-bearing chickens were studied both histochemically and biochemically for the distribution and content of the acid and alkaline phosphomonoesterases.
2. A modification of the original methods of Gomori consisting of mounting the tissue sections on cover glasses and incubating them lying flat in the substrate solutions contained in Petri dishes, gave better results for the histochemical study of both acid and alkaline phosphatases.
3. It was found by trial and error that 18-20 hours incubation for acid phosphatase and 12-16 hours incubation for alkaline phosphatase are the most suitable time of incubation for the study of these enzymes in chicken tissues.
4. It was found that acid phosphatase is present in nearly all nuclei. Alkaline phosphatase has not such a wide-spread distribution, occurring mainly in the connective tissue stroma of organs.
5. The co-relation curve between the density and time of incubation of acid and alkaline phosphomonoesterases of the normal chicken kidney has been plotted.
6. There is no apparent co-relation between the degree of malignancy and the content of acid or alkaline phosphatase.

7. /

7. The alkaline phosphatase activity of the GRCH/15 sarcoma is fairly high compared with the activity of the host's organs with the exception of liver and kidney. The acid phosphatase activity of Rous sarcoma is low compared with other tissues of the host.
8. The GRCH/15 (dibenzanthracene induced) tumour-bearing birds were usually found to be affected with liver cirrhosis, and the alkaline phosphatase content of the organ was greatly reduced. The reason for this is discussed.
9. Some of the suggested physiological functions of the phosphomonoesterases received further confirmation in the present histochemical study.
10. Discrepancies between the present findings and those recorded by previous workers have been mentioned and discussed briefly.

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VI. Acknowledgements.

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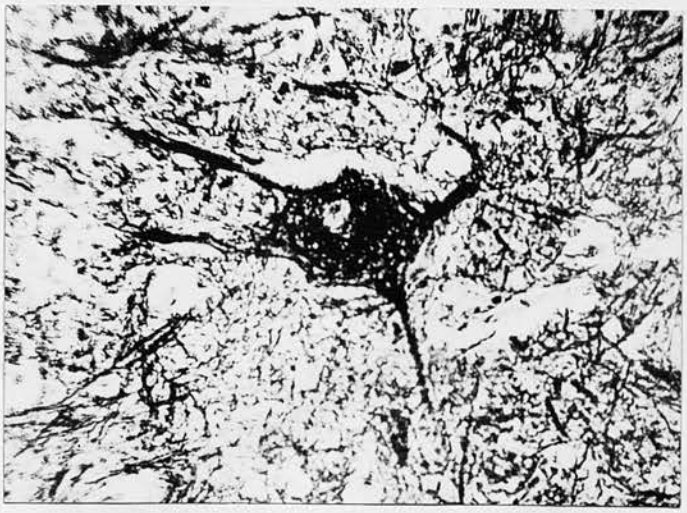

Fig.1. Section of a healthy chick's brain showing unequal distribution of acid phosphatase in motor neurons. Neurofibrils were clearly stained. X 370.

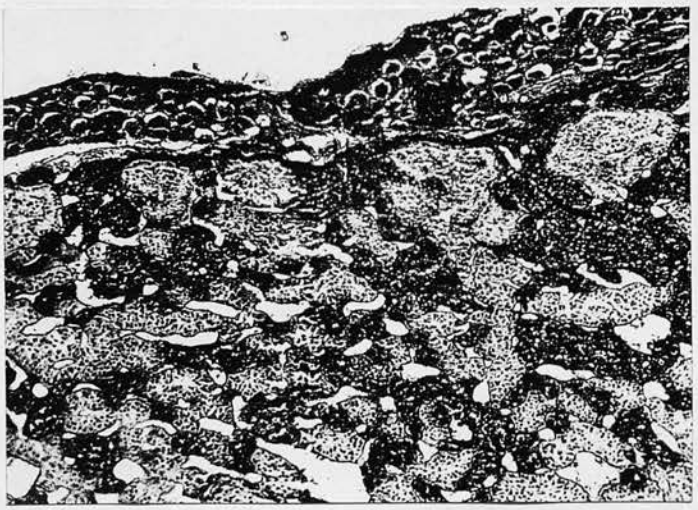

Fig.2. Section of a healthy chick's adrenal gland together with its neighbouring sympathetic ganglion (upper portion). The nerve ganglion is very rich in acid phosphatase both in nerve cells and connective tissue. The adrenal gland is chiefly rich in cortical cells. X 85.

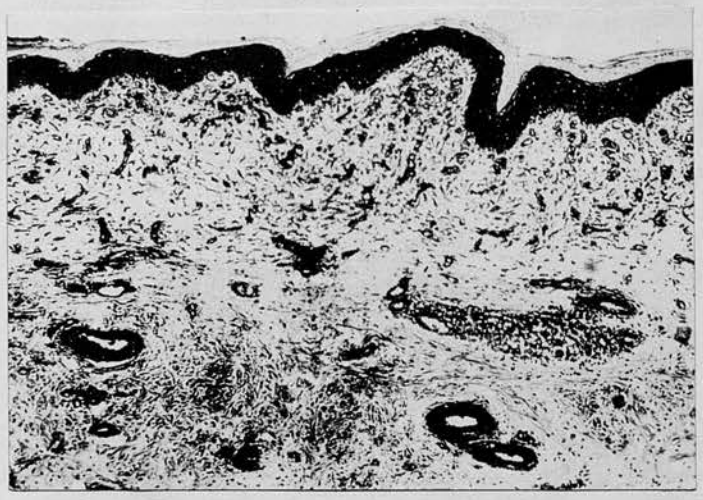

Fig.3. Section of a healthy chick's comb showing that the acid phosphatase is very rich in the Malpighian layer of the skin, the blood vessels, and the lymphoid cells. X 85.

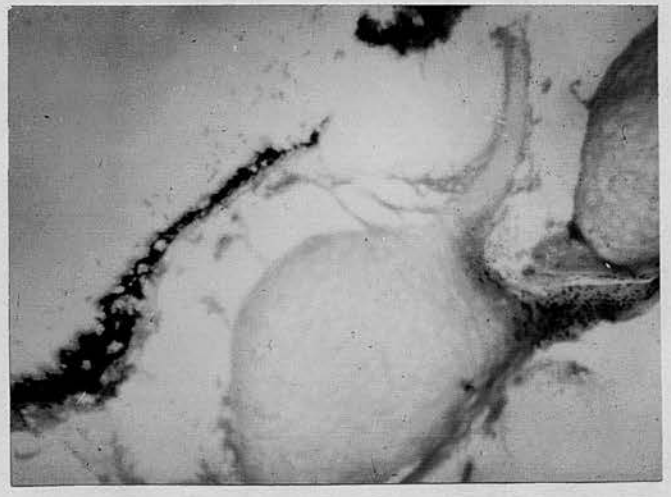

Fig.4. Longitudinal section of a healthy chick's trachea showing that the acid phosphatase is very rich in the epithelium, moderate in the ligament, and nearly none in the cartilage. X 135.

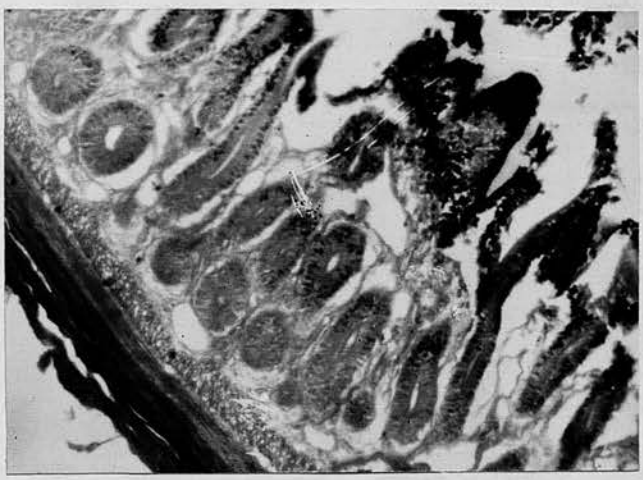

Fig. 5. Cross section of a healthy chick's duodenum showing that the acid phosphatase is chiefly limited to the epithelial cells. X 135.

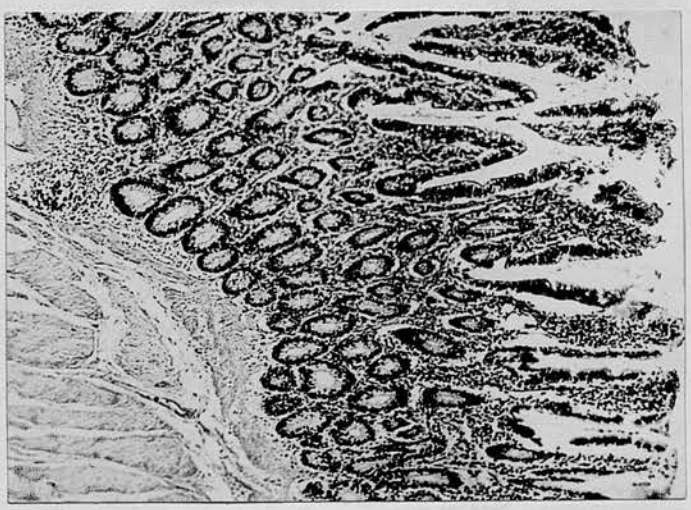

Fig. 6. Section of the ileo-caecal junction of a healthy chick showing that the acid phosphatase is rich in epithelial cells and glandular cells, lesser amount in lymphoid cells. X 85.

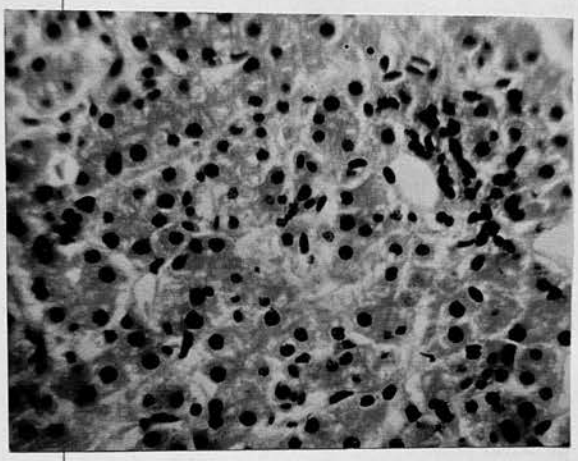

Fig. 7. Section of a healthy chick's liver showing the distribution of acid phosphatase is chiefly in all the nuclei. X 370.

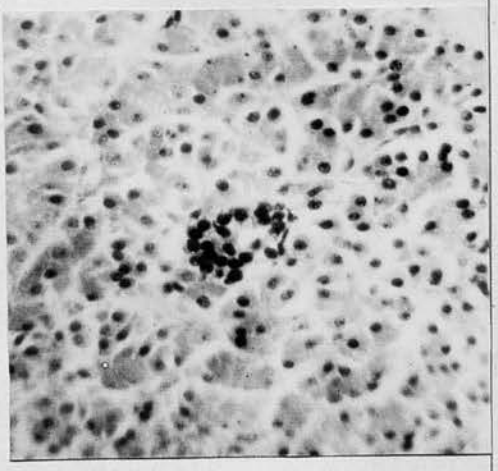

Fig. 8. Section of a healthy chick's pancreas showing the nuclei of the islet of Langerhans are richer in acid phosphatase than the exocrine cells. X 370.

Fig. 9. Section of a healthy chick's pineal body showing the richness of acid phosphatase. X 135.

Fig. 10. Section of a healthy chick's pituitary gland (pars nervosa) showing the acid phosphatase is chiefly in the nerve cells and the fibrous tissue. X 135.

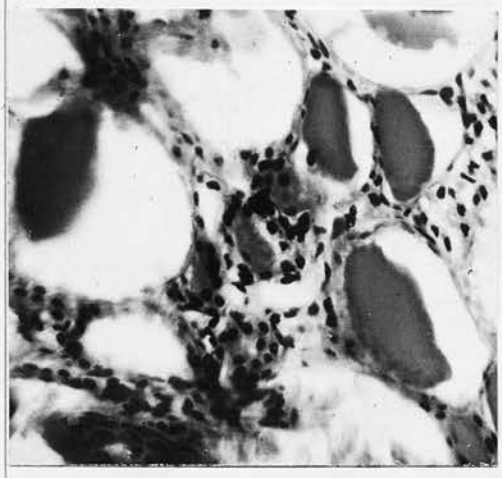

Fig. 11. Section of a healthy chick's thyroid gland showing the acid phosphatase is rich in the nuclei of the follicular epithelium. X 370.

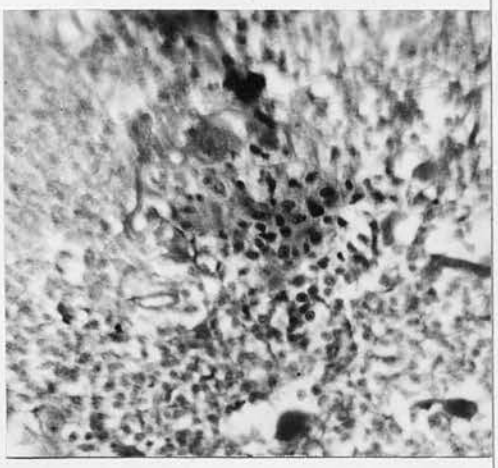

Fig. 12. Section of a healthy chick's thymus showing the nuclei of the Hassall's body containing more acid phosphatase than thymocytes. X 370.

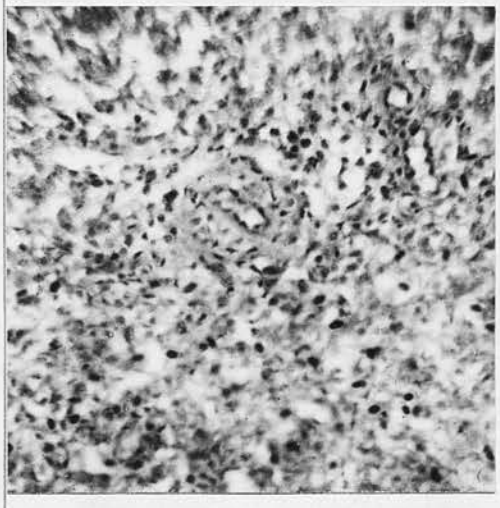

Fig. 13. Section of a healthy chick's spleen showing the acid phosphatase is chiefly in the nuclei of the endothelial cells of the sinusoids and blood vessels. X 370.

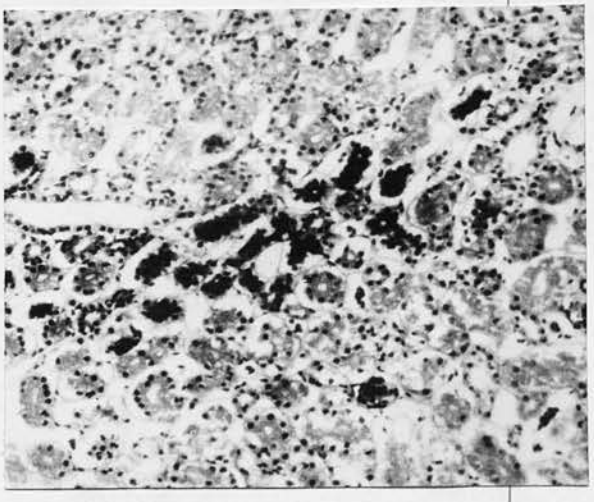

Fig. 14. Section of a healthy chick's kidney showing that acid phosphatase is rich in the nuclei of renal tubules. X 135.

Fig. 15. Section of a healthy chick's Bursa Fabricii showing acid phosphatase in the nuclei of lymphoid cells and epithelial cells. X 135.

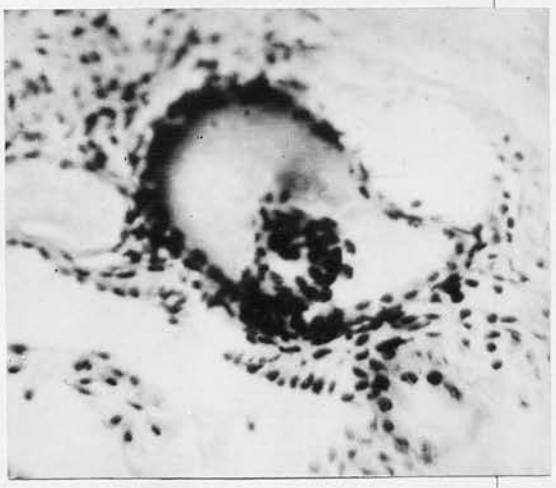

Fig. 16. Section of a healthy chick's Graafian follicle showing acid phosphatase is especially rich in the follicular cells. X 370.

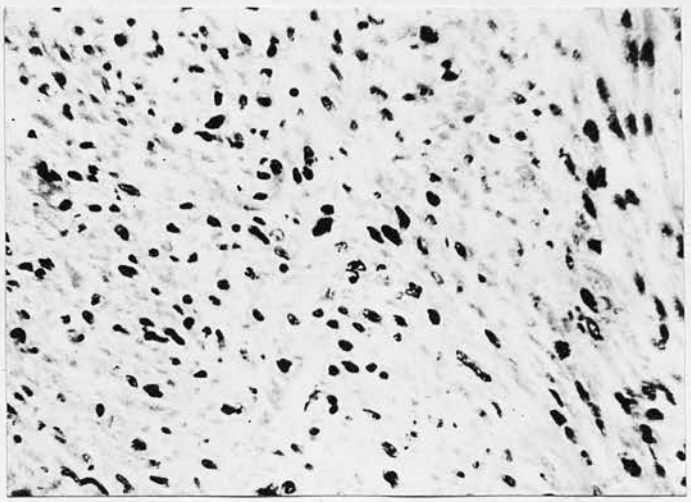

Fig. 17. Section of Rous No. 1 sarcoma of a chick showing acid phosphatase is chiefly limited to the nuclei of both tumour cells and stromal cells. Some mitotic figures were clearly stained. X 370.

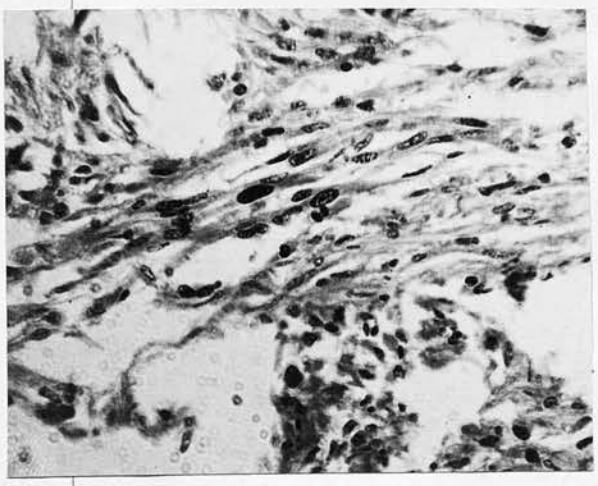

Fig. 18. Section of G.R.C.H./15 1.2.5.6. dibenzanthracene-induced fibro-sarcoma of chicken, showing the acid phosphatase is chiefly in the nuclei. Many mitotic figures were heavily stained. X 370.

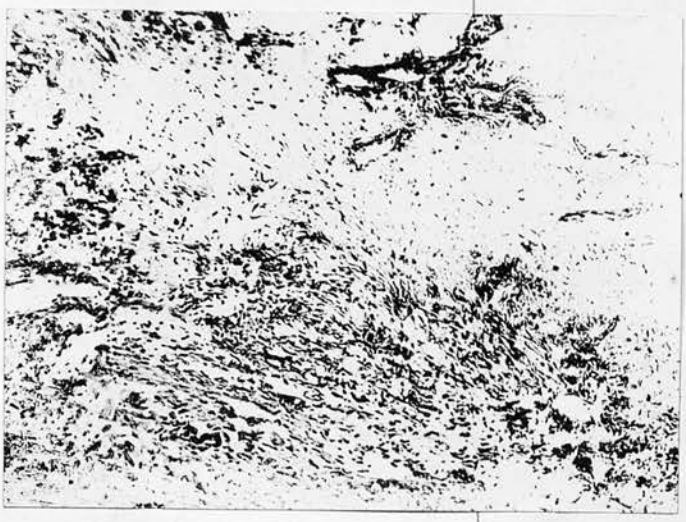

Fig. 19. Section of adeno-carcinoma of chicken showing acid phosphatase in the nuclei of the tumour cells and stromal cells. X 85.

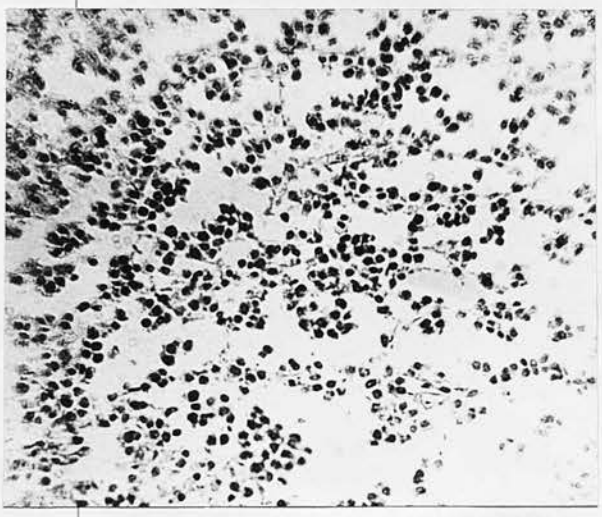

Fig. 20. Section of a spontaneous lymphocytoma of chicken showing acid phosphatase chiefly in the nuclei of the tumour cells. X 370.

Fig. 21. Section of a spontaneous fibro-leiomyoma of a chicken showing acid phosphatase in the nuclei of both tumour cells and stromal cells. X 85.

Fig. 22. Section of a healthy chick's adrenal gland (lower portion) and its neighbouring sympathetic ganglion (upper portion), showing that alkaline phosphatase is profuse in the ganglion and fibrous capsule of adrenal gland. X 135.

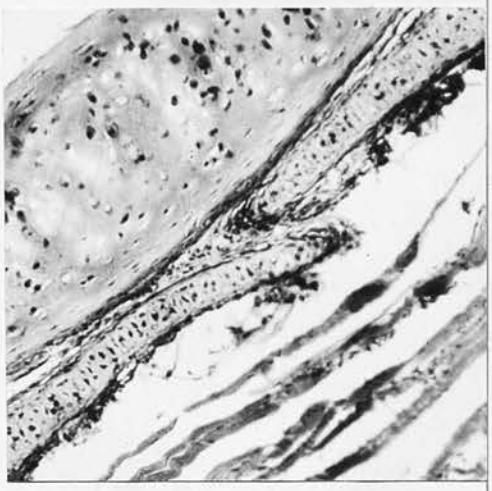

Fig. 23. Longitudinal section of a healthy chick's trachea showing moderate amount of alkaline phosphatase in the nuclei of the cartilage cells and fibrous tissue. X 135.

Fig. 24. Section of a healthy chick's lung showing the alkaline phosphatase is especially rich in the endothelial cells of the blood vessels, red blood cells, and epithelial cells of the bronchioles. X 135.

Fig. 25. Section of a healthy chick's Oesophagus showing alkaline phosphatase is extremely rich in the stratum germinativum of the epithelium, lesser amount in the nuclei of fibrous tissue and the mucous glands. X 85.

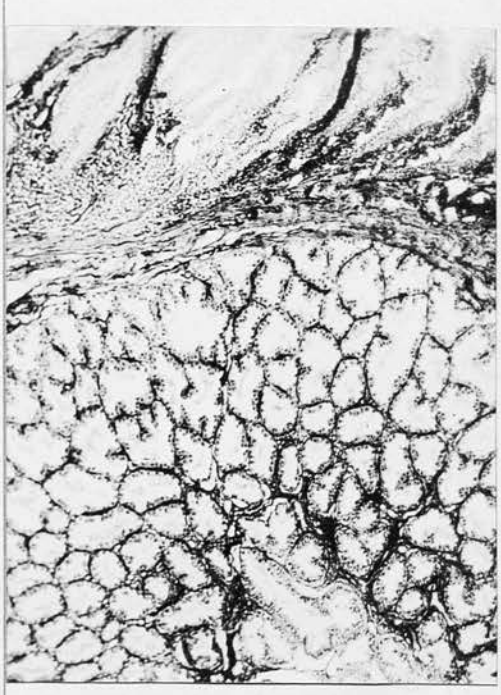

Fig. 26. Section of a healthy chick's glandular stomach showing alkaline phosphatase is richer in the lymphoid cells, the nuclei of glandular cells, and esp. the reticular fibres which situated between glandular lobules. X 85.

Fig. 27. Section of a healthy chick's duodenum showing alkaline phosphatase exists in large amount in the villi and lymph nodule. X 85.

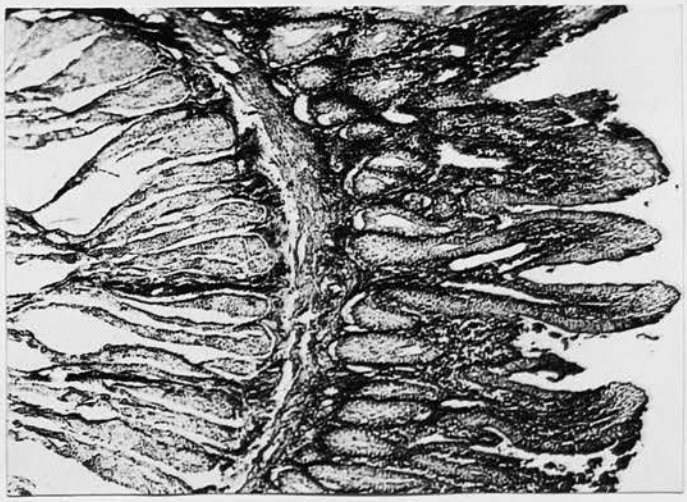

Fig. 28. Section of a healthy chick's ileo-caecal junction showing the alkaline phosphatase is rich in the villi (esp. goblet cells), the lymphoid cells, the basement membrane of the tubular gland, and the reticulum fibres. X 85.

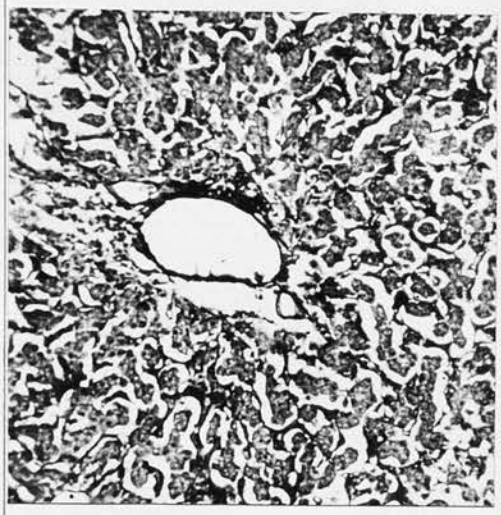

Fig. 29. Section of a healthy chick's liver showing the alkaline phosphatase is rich in the endothelial cells of the blood vessàls and the reticulo-endothelial cells. X 135.

Fig. 30. Section of a healthy chick's pancreas showing alkaline phosphatase exists chiefly in the nuclei of glandular cells, richer in blood capillary wall. X 135.

Fig. 31. Section of a healthy chick's pineal body showing the richness of alkaline phosphatase. X 135.

Fig. 32. Section of a healthy chick's pituitary gland showing the richness of alkaline phosphatase. (Pars nervosa) X 135.

Fig. 33. Section of a healthy chick's thyroid gland (upper part) and thymus showing alkaline phosphatase is profuse in the thyroid gland, moderate in thymus, and richer in Hassall's bodies than in thymocytes. X 135.

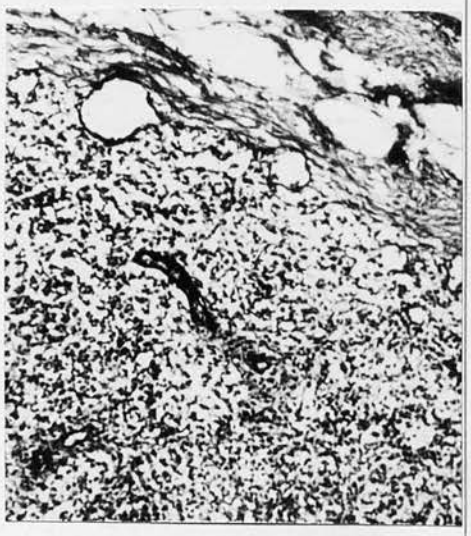

Fig. 34. Section of a healthy chick's spleen showing the alkaline phosphatase is richer in the endothelium of blood vessels and sinusoids, and richer in germinal centres than surrounding area. X 135.

Fig. 35. Section of a healthy chick's kidney showing the richness of alkaline phosphatase in the renal tubules, esp. the brush-border of the proximal convoluted tubules. X 135.

Fig. 36. Section of a healthy chick's Bursa Fabricii showing alkalin e phosphatase exists chiefly in the nuclei of epithelial cells, lymphoid cells, and reticular fibres. X 135.

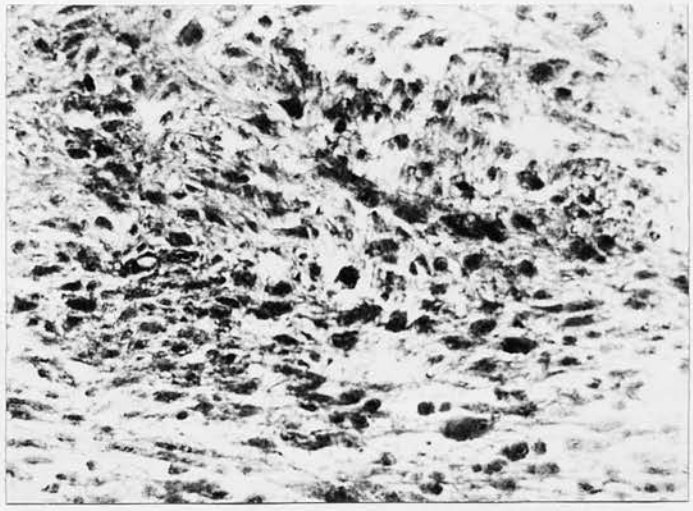

Fig. 37. Section of a Rous No. 1 sarcoma showing alkaline phosphatase is mainly present in the nuclei. Some mitotic figures are clearly visible. X 370.

Fig. 38. Section of G.R.C.H./15 I. 2. 5. 6. dibenzanthracene induced fibro-sarcoma of chicken showing that alkaline phosphatase is present in both the interstitial tissue and parenchymatous tissue. X 85.

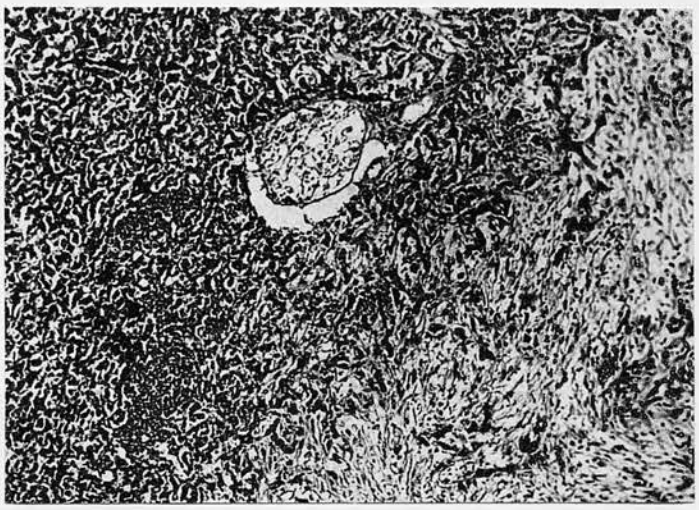

Fig. 39. Section of a metastatic Rous No.1 tumour (lower right portion) in the liver of a chicken showing that acid phosphatase is chiefly limited to the nuclei of both the liver tissue and the tumour tissue. X 85.

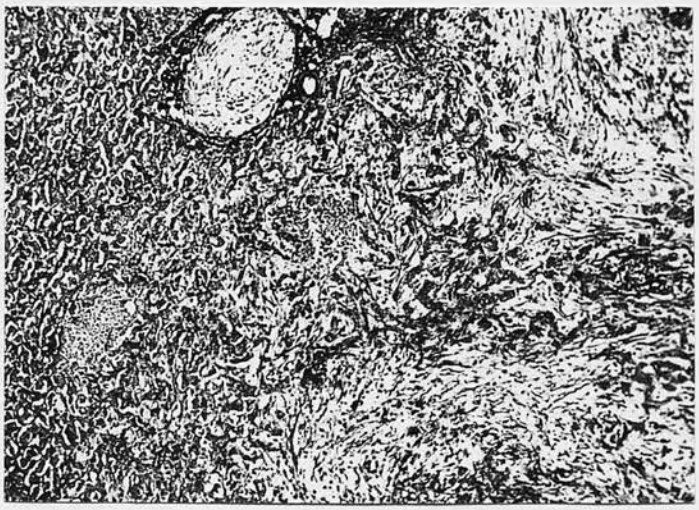

Fig. 40. Section of a metastatic Rous No.1 tumour (lower right portion) in the liver of a chicken showing that alkaline phosphatase is especially rich in the reticulo-endothelial cells network of the liver tissue (upper left) and the reticulum fibres of the tumour. X 85.

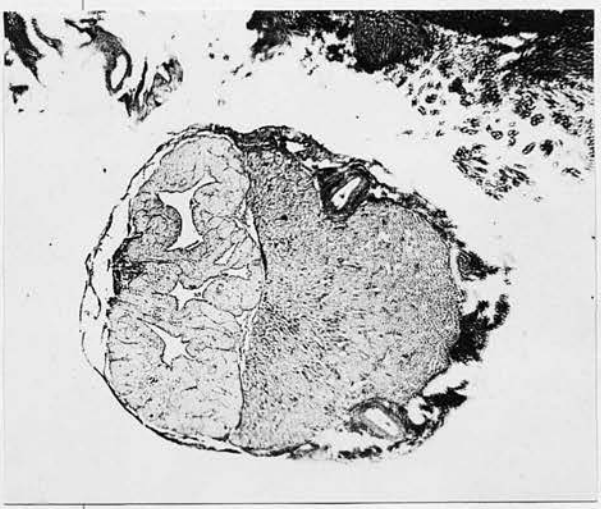

Fig. 41. Sagital section of hypophysis showing alkaline phosphatase is present in the fibrous capsule, and blood vessels, less amount in the parenchymatous cells. X 22. Counter-stained with Mason's trichrome staining.

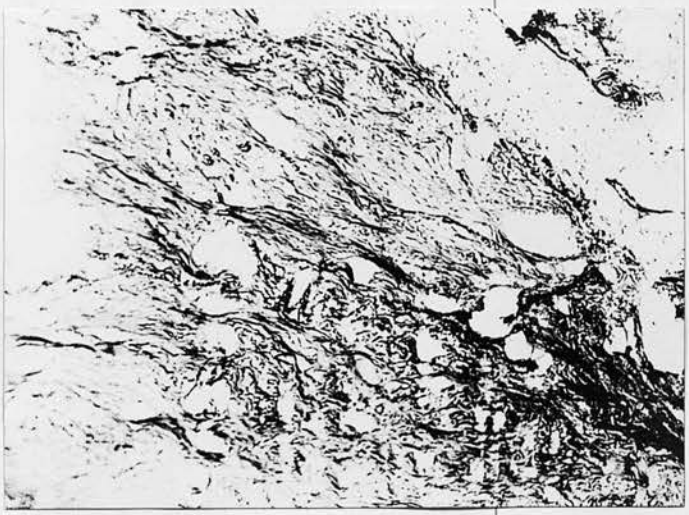

Fig. 42. Section of a spontaneous adenocarcinoma showing that alkaline phosphatase is chiefly limited to interstitial fibrous tissue. and some nuclei. X 85.

Fig. 43. Section of a spontaneous fibroleiomyoma showing that alkaline phosphatase is rich in the reticulum fibres and the endothelium of the blood vessels. X 85.

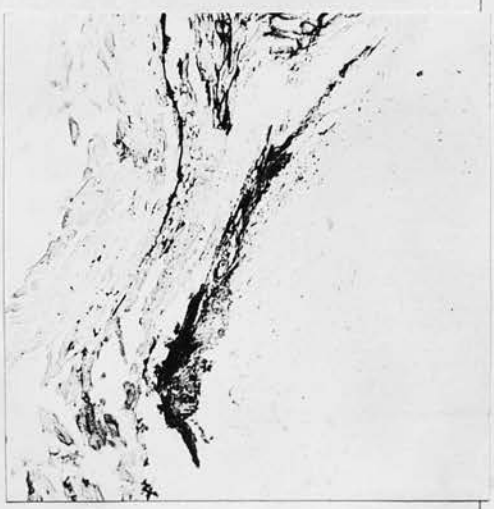

Fig. 44. Section of a spontaneous histiocytic sarcoma showing alkaline phosphatase is rich in the fibrous capsule, nearly none in the tumourous tissue. X 85.

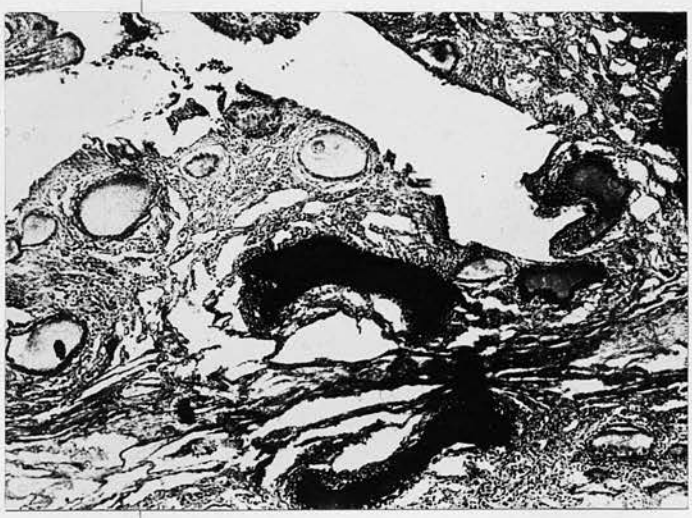

Fig.45. Section of a normal ovary showing increasing amount of acid phosphatase activity in accordance with the degree of maturity of the ovum. X 85.

Fig.46. Section of the same normal ovary showing no obvious difference in alkaline phosphatase activity in different Graafian follicles. It is chiefly in follicular cells, theca, and interstitial tissue. X185

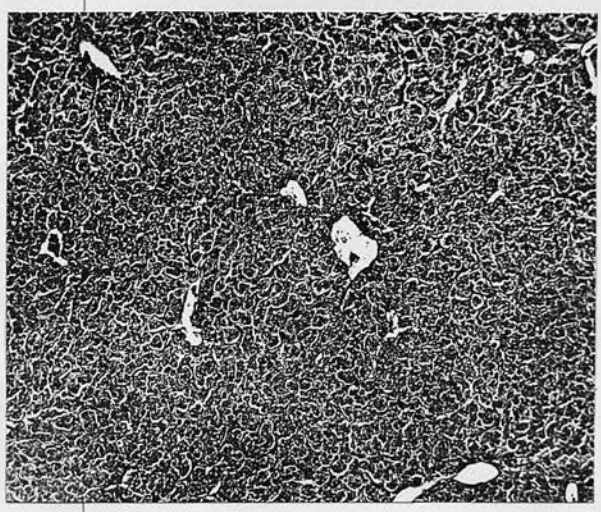

Fig.47. Section of normal liver stained with Masson's Ponceau-Acid Fuchsin-Light Green Method showing the scarcity of connective tissue and the normal arrangement of liver cells and reticulo-endothelial network. X 85.

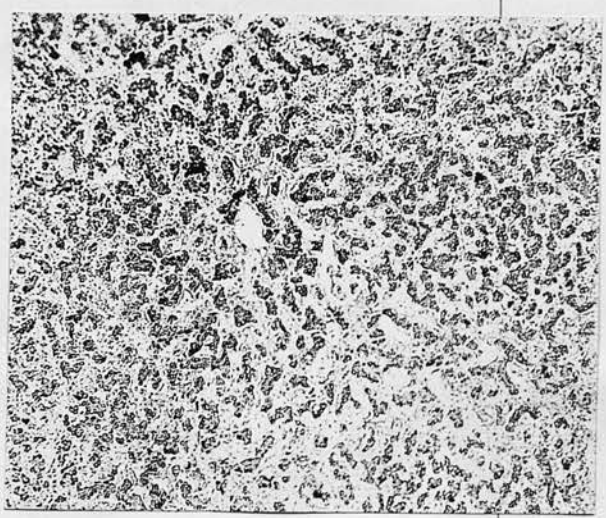

Fig.48. Section of the cirrhotic liver of a chick bearing GRCH/15 tumour stained with Masson's Ponceau-Acid Fuchsin-Light Green Method showing the liver cells were scattered into small groups by the hyperplastic connective tissue. X 85.

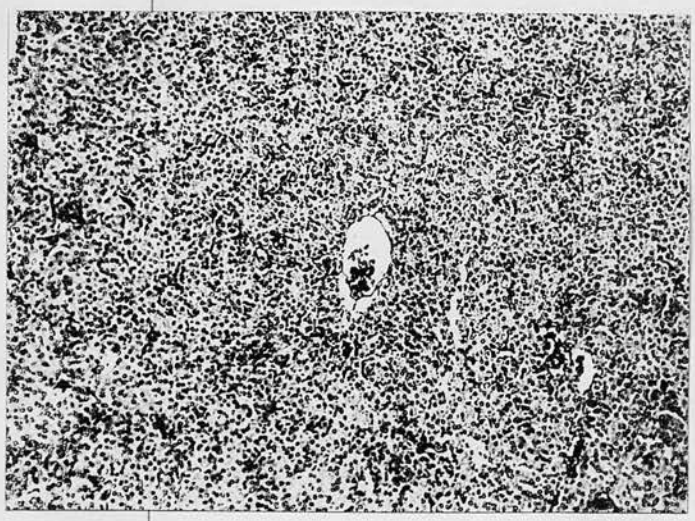

Fig.49. Section of normal liver showing acid phosphatase is evenly distributed in all nuclei and the cytoplasm of some of the liver cells. X 85.

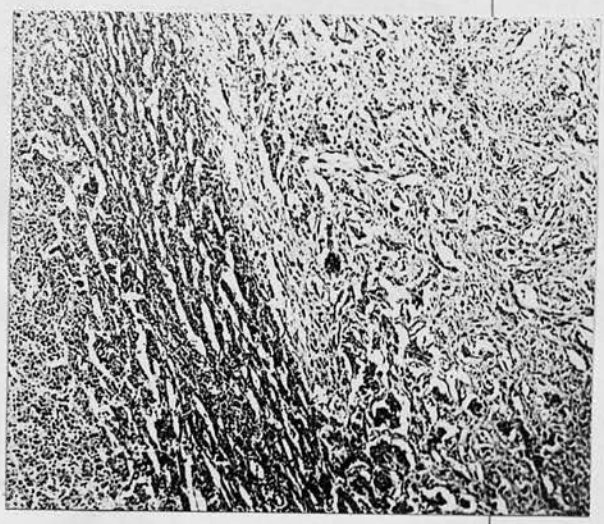

Fig.50. Section of a piece of liver bearing a GRCH/15 tumour (right half) showing lower acid phosphatase activity in the tumour tissue and the fibrous capsule. X 85.

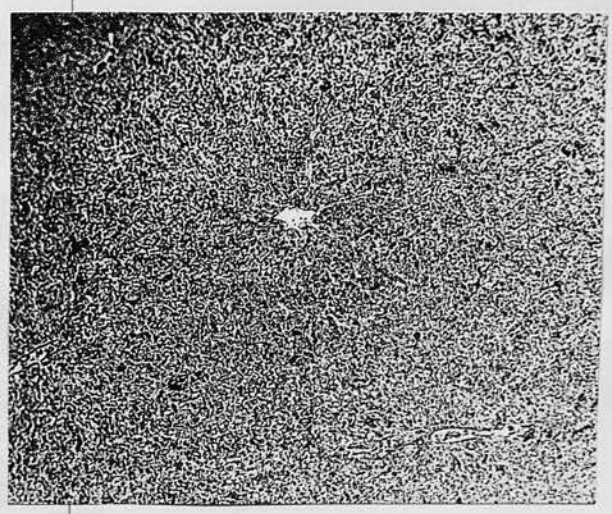

Fig.51. Section of normal liver showing alkaline phosphatase is richer in the reticulo-endothelial network, endothelium, and nuclear membrane of liver cells. X 85.

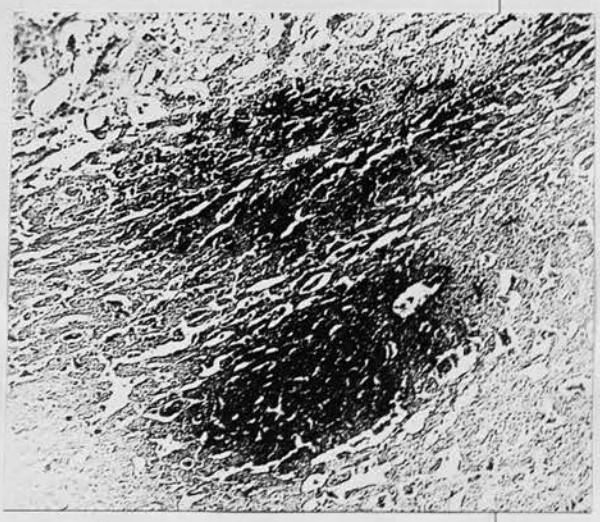

Fig.52. Section of the same piece of liver bearing GRCH/15 tumour (upper left) showing alkaline phosphatase is limited to the surrounding area of several central veins only. X 85.